APPLICATION FOR PATENT

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Title: METHOD OF IDENTIFYING PEPTIDES CAPABLE OF BINDING TO MHC MOLECULES, PEPTIDES IDENTIFIED THEREBY AND THEIR USES

This Application claims the benefit of priority from U.S. Provisional Patent Application No. 60/290,958, filed May 16, 2001.

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FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to a method of identifying peptides of a desired origin and which are capable of binding to MHC molecules of a particular haplotype; peptides identified by the method; pharmaceutical compositions containing the peptides, databases describing the peptides and the use of the peptides in vaccination.

The following abbreviations are used herein: MHC, Major Histocompatibility Complex; β2m, β2-microglobulin; ESI, electrospray ionization; MS, mass spectrometry; m/z, mass charge ratio; CID, collision induced disintegration; MS/MS, tandem mass spectrometry; MTDM, DNA methyl transferase; FAS, fatty acid synthase; CTL, cytotoxic T lymphocytes; mAbs, monoclonal antibodies.

The MHC serves as a shuttle to transport and display peptide antigens on the surface of cells as an indication to the immune system of the health state of the cells. Each individual has at most six different MHC class-I haplotypes, out of the hundreds known. MHC bound peptides, i.e., peptides bound to, and presented in context of, MHC molecules, originate from proteolysis of most of the proteins expressed in the cells. Therefore, unique sets of MHC bound peptides are displayed by each of the different MHC haplotypes according to the protein expression and degradation schemes of the cells and according to the peptide binding motifs of the MHC molecules (reviewed in [1]). Therefore, thousands of different peptides are presented by the different MHC class-I haplotypes and each of the peptides is presented in vastly differing copies per cell [2]. When cells become infected, some of the presented peptides are derived from the pathogen's proteins, and so indicate to circulating T-cells to kill the diseased cells and prevent the spread of the disease.

Each MHC haplotype recognizes the peptides through a broadly defined consensus motif of peptide's amino acids strategically positioned to serve as anchors to the appropriate binding pockets on the MHC molecule. The binding motifs of many of the MHCs haplotypes were first established by pool Edman sequencing of unfractionated peptide mixtures eluted from immunoaffinity purified MHC molecules [3, 4]. The consensus was further extended by direct biochemical analysis of individual peptides separated by

reversed phase chromatography and analyzed by tandem mass spectrometry [2, 5, 6], reviewed in [7].

MHC bound peptides derived from cancer specific or associated proteins or antigens were extensively searched for, with the goal of finding among them peptide candidates for development of anti-cancer vaccines. A number of such tumor specific peptides were already identified and some were successfully tested as anti-cancer vaccines for human treatment, most notably for immunotherapy of melanoma [8, 9]. Three main approaches were extensively used for the identification of such MHC bound peptides [10]. The genetic approach involves transfection of cDNA libraries, made from tumor cells, into cells that present the MHC allele of interest. The clones of transfected cells that stimulated CTL lines against the tumor cells were selected as the source for the tumor antigen and the genes were further fragmented to isolate the regions of the genes that encode the particular immunogenic peptide [11]. The second approach is based on exploiting the known consensus binding motifs of the MHC haplotype of interest to scan sequences of known protein "*in silico*" and to predict putative MHC bound peptides that fit this consensus [12]. For successful prediction, these consensus motifs should be *a priori* well established, which is not the case for many of the MHC haplotypes [13]. The drawback of this approach is its reliance on chemical synthesis of a large number of peptides, only few of which end up being useful. The biochemical, third approach, involves the

fractionation of the MHC bound peptides by chromatography, assaying the fractions for immuonoglogical activity and sequencing the individual peptides in the active fractions [2, 5]. The biochemical approach is the only possible way to identify post-translationally modified peptides, not always predictable from the protein sequences [14-16]. The biochemical approach depends on the availability of advanced mass spectrometry, needed for analyzing the available minute amounts of peptides that are present at very complex mixtures (reviewed in [7]).

All these approaches for identifying MHC bound peptides eventually rely on chemical synthesis of the peptides of interest to test their capacity to bind to the MHC molecule by stabilization of empty MHC molecules on cell surface [17], and their potential to elicit an immune response by tetramer assays [18], ELISPOT [19] and elicitation CTL responses when presented on cells [20].

Currently, sequencing and identification of individual MHC bound peptides by the direct biochemical approach is most effectively performed by use of tandem mass spectrometry. The peptides are resolved by reversed phase chromatography and the eluting peptides are collected, assayed for biological activity and sequenced, most often by electrospray tandem mass spectrometry [2, 5, 21]. Comparing the patterns of MHC bound peptides recovered from healthy and infected cells helps to identify disease related peptides [22]. Mass spectrometry is advantageous for such analysis due to its accuracy, speed of

analysis, its ability to analyze complex mixtures of peptides and its high sensitivity [7]. The biochemical analysis involves the purification of the MHC molecules with their bound peptides by immunoaffinity chromatography using mAbs specific for the native MHC [2]. To this end, the cells are solubilized with detergents, the desired MHC molecules are purified with their MHC bound peptides and the MHC bound peptides are recovered by denaturation and ultra-filtration. However, once the cells are disrupted by the detergents, the MHC molecules become contaminated by cellular debris and detergents which complicates the subsequent ESI-MS/MS analysis. Moreover, such immunoaffinity purification of desired MHC haplotypes is possible only when specific mAbs are available, whereas for many MHC haplotypes such mAbs are presently unavailable.

There is thus a widely recognized need for, and it would be highly advantageous to have, a method for identifying MHC bound peptides devoid of the above limitations.

SUMMARY OF THE INVENTION

While conceiving the present invention, it was hypothesized that MHC bound peptides presented within the context of different MHC haplotypes on cells of different tissues or tumor origins can be biochemically identified by transforming the cells to express and secrete soluble MHC molecules of the

different MHC haplotypes, with the aim of biochemically identifying the MHC bound peptides that bind to the soluble MHC molecules. Should this approach be successful, it solves three major problems associated with the prior art biochemical approach. First, although not excluded, there is no need for specific mAbs per each tipe of MHC, rather general mAbs such as W6/32 (anti HLA-A, B and C) can be used to isolate the sMHC and hence the MHC bound peptides from the growth medium in which the cells are grown. Second, while the prior art approach relies on native MHC molecules, different MHC haplotypes directing the expression of different soluble MHC molecules can potentially be used for each of the cells, to thereby increase the repertoire of MHC bound peptides which can be used as, for example, anti-cancer vaccines. Third, since the cells are not disrupted and further since there is no use of detergents, the sMHC molecules do not become contaminated by cellular debris and detergents which otherwise complicates the subsequent ESI-MS/MS analysis.

According to one aspect of the present invention there is provided a method of identifying peptides originating from a particular cell type and being capable of binding to MHC molecules of a particular haplotype, the method comprising obtaining a cell type expressing a soluble and secreted form of the MHC molecules of the particular haplotype; collecting the soluble and secreted form of the MHC molecules of the particular haplotype; and analyzing peptides bound to the soluble and secreted form of the MHC molecules of the

particular haplotype, thereby identifying the peptides originating from the particular cell type and being capable of binding to MHC molecules of the particular haplotype.

According to further features in preferred embodiments of the invention described below, the cell type is a cancer cell.

According to still further features in the described preferred embodiments the cell type is a cancer cell line.

According to still further features in the described preferred embodiments the cell type is a virus infected cell or cell line.

According to still further features in the described preferred embodiments the cell type is a cell involved in a development and/or progression of an autoimmune diseases.

According to another aspect of the present invention there is provided a method of identifying peptides originating from at least one protein of interest and being capable of binding to MHC molecules of a particular haplotype, the method comprising obtaining cells co-expressing the at least one protein of interest and a soluble and secreted form of the MHC molecules of the particular haplotype; collecting the soluble and secreted form of the MHC molecules of the particular haplotype; analyzing peptides bound to the soluble and secreted form of the MHC molecules of the particular haplotype; and identifying peptides originating from the at least one protein of interest and being capable of binding to MHC molecules of the particular haplotype.

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According to further features in preferred embodiments of the invention described below, the protein of interest in natively expressed by the cells.

According to still further features in the described preferred embodiments the at least one protein of interest in expressed by the cells following transformation of the cells with nucleic acid encoding for the at least one protein of interest.

According to still further features in the described preferred embodiments the at least one protein of interest includes a tumor associated antigen.

According to still further features in the described preferred embodiments the at least one protein of interest includes a cytokine.

According to still further features in the described preferred embodiments the at least one protein of interest includes a protein of a pathogen.

According to still further features in the described preferred embodiments the soluble and secreted form of the MHC molecules include a polypeptide encoded by exons 5 to 8 of a murine mutant $Q10^{b}$.

According to still further features in the described preferred embodiments analyzing the peptides bound to the soluble and secreted form of the MHC molecules of the particular haplotype is by mass spectrometry, mass charge ratio and collision induced disintegration.

According to still further features in the described preferred embodiments identifying peptides originating from the at least one protein of interest and being capable of binding to MHC molecules of the particular haplotype is by comparison to a protein database.

According to another aspect of the present invention, there is provided an electronic data storage device, storing, in a retrievable form, a plurality of sequences of peptides identified by the methods described herein.

According to still another aspect of the present invention, there is provided a kit comprising a plurality of individual containers, each of the plurality of individual containers containing at least one peptide identified by the methods described herein.

According to yet another aspect of the present invention there is provided a method of identifying peptides originating from cancer associated proteins and being capable of binding to MHC molecules of a particular haplotype, the method comprising obtaining a cancer cell type expressing a soluble and secreted form of the MHC molecules of the particular haplotype; collecting the soluble and secreted form of the MHC molecules of the particular haplotype; analyzing peptides bound to the soluble and secreted form of the MHC molecules of the particular haplotype; and identifying peptides originating from cancer associated proteins and being capable of binding to MHC molecules of the particular haplotype.

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According to still another aspect of the present invention there is provided a method of identifying peptides originating from cells participating in the development and/or progression of an autoimmune disease and being capable of binding to MHC molecules of a particular haplotype, the method comprising obtaining cells participating in the development and/or progression of the autoimmune disease and expressing a soluble and secreted form of the MHC molecules of the particular haplotype; collecting the soluble and secreted form of the MHC molecules of the particular haplotype; analyzing peptides bound to the soluble and secreted form of the MHC molecules of the particular haplotype; and identifying peptides originating from proteins participating in the development and/or progression of the autoimmune disease and being capable of binding to MHC molecules of the particular haplotype.

According to an additional aspect of the present invention there is provided a method of identifying peptides originating from virus infected cells and being capable of binding to MHC molecules of a particular haplotype, the method comprising obtaining virus infected cells expressing a soluble and secreted form of the MHC molecules of the particular haplotype collecting the soluble and secreted form of the MHC molecules of the particular haplotype; analyzing peptides bound to the soluble and secreted form of the MHC molecules of the particular haplotype; and identifying peptides originating from the virus and being capable of binding to MHC molecules of the particular haplotype.

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According to yet an additional aspect of the present invention there is provided a method of identifying peptides originating from a particular cell type characterized by at least one of the following (i) cell over-expressing at least one protein; (ii) cells characterized by induced mutations; (iii) cells of metastases; (iv) normal or transformed cells expressing cell surface proteins, the peptides being capable of binding to MHC molecules of a particular haplotype, the method comprising obtaining cells of the particular cell type expressing a soluble and secreted form of the MHC molecules of the particular haplotype; collecting the soluble and secreted form of the MHC molecules of the particular haplotype; analyzing peptides bound to the soluble and secreted form of the MHC molecules of the particular haplotype; and identifying peptides originating from the particular cell type and being capable of binding to MHC molecules of the particular haplotype.

According to still an additional aspect of the present invention there is provided an electronic data storage device, storing, in a retrievable form, a plurality of peptides being arranged at least according to their association with a pathology and further according to their ability of binding to MHC molecules of a particular haplotype.

According to a further aspect of the present invention there is provided an electronic data storage device, storing, in a retrievable form, a plurality of peptides being arranged at least according to their association with a protein of

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interest and further according to their ability of binding to MHC molecules of a particular haplotype.

According to yet a further aspect of the present invention there is provided a method of eliciting an immune response against a protein of interest in a subject having a particular MHC haplotype, the method comprising determining the subject's particular MHC haplotype; and administering to the subject an effective amount of at least one peptide derived from the protein of interest and which is capable of binding to MHC molecules of the particular haplotype.

According to still a further aspect of the present invention there is provided a method of treating a pathology by eliciting an immune response against a protein of interest in a subject having a particular MHC haplotype, the method comprising determining the subject's particular MHC haplotype; and administering to the subject a therapeutic effective amount of at least one peptide derived from the protein of interest and which is capable of binding to MHC molecules of the particular haplotype.

According to an additional aspect of the present invention, there is provided a method of eliciting an immune response against a protein of interest in a subject, the method comprising using an individualized in vitro assay for determining an immune reactivity of an immune system of the subject to a plurality of peptides derived from the protein of interest; and administering to the subject an effective amount of at least one peptide derived from the protein

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of interest and which is capable of inducing predetermined sufficient immune reactivity.

According to further features in preferred embodiments of the invention described below, administering to the subject the therapeutically effective amount of the at least one peptide is accompanied by presenting the at least one peptide in context of an antigen presenting cell.

According to still an additional aspect of the present invention, there is provided a peptide selected from the group consisting of SEQ ID NOs:4-6, 10-14, 19-21, 23-37, 44-88, 90-141, 143-144, 146-173, 175-189 and 191-195, all of which were never reported to bind MHC molecules.

According to still an additional aspect of the present invention, there is provided a peptide selected from the group consisting of SEQ ID NOs: 5, 9, 10 and 25.

According to yet an additional aspect of the present invention, there is provided a peptide selected from the group consisting of SEQ ID NOs:13, 20, 23 and 24.

According to another aspect of the present invention, there is provided a pharmaceutical composition comprising, as an active ingredient, at least one of the peptides described herein, and a pharmaceutically acceptable carrier. Preferably, the at least one of the peptides is presented in context of an antigen presenting cell.

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According to further features in preferred embodiments of the invention described below, the peptide comprises at least one modification rendering peptides more stable in a body and/or more immunogenic.

According to still further features in the described preferred embodiments the at least one modification is selected from the group consisting of peptoid modification, semipeptoid modification, cyclic peptide modification, N terminus modification, C terminus modification, peptide bond modification, backbone modification and residue modification.

The present invention successfully addresses the shortcomings of the presently known configurations by providing a novel method for the identification of MHC bound peptides.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more

detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIGs. 1A-C demonstrate the purification of soluble MHC from cancer cells. Soluble MHCs was purified by immunoaffinity from the growth medium of $5x10^7$ transfected cells. Purified proteins were analyzed on 10% SDS-PAGE and stained with Coomassie blue. (1A) Purification of HLA-A2/Q10^b from MCF-7 cells, (1B) Purification of sHLA-A2 from UCI-101 cells, (1C) Purification of sHLA-B7 from UCI-107 cells.

FIGs. 2A-C show a typical nano-capillary reversed-phase chromatography of MHC bound peptides purified from soluble MHC from $5X10^7$ MCF-7 breast cancer cells. (2A) The total-ion-current chromatogram (TIC). (2B) Mass spectrum taken at the time point of 33.3 minutes. (2C) Spectrum of the collision-induced-disintegration (CID) of the dominant peptide in 2B having a m/z of 1028.5 that eluted at the 33.3 minutes. The putative MHC peptide GLIEKNIEL (SEQ ID NO:13) was identified to originate from DNA-methyl transferase.

FIGs. 3A-B show a comparison of the chromatographs, the MS and the CID spectra of the synthetic peptide: GLIEKNIEL (SEQ ID NO:13) of the DNA methyl transferase (3A) with those of the peptide m/z=1028.5 (SEQ ID NO:13) from the breast cancer line MCF-7 (3B).

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FIGs. 4A-D demonstrate the evaluation of the correctness of the MAGE-B2 peptide p1091 (GVYDGEEHSV, SEQ ID NO:20) by comparing the retention time and CID spectra of the synthetic peptide (4A) to that of the natural peptide m/z=1091.4 (SEQ ID NO:20) from the ovarian cancer line UCI-107 (4B). (4C) Evaluation of the binding affinity of peptide p1091 (SEQ ID NO:20) to HLA-A2 by reconstituting it into cells surface empty MHC of the RMA-S-HHD cells as assayed by FACS analysis. (4D) The homology between this MAGE-B2 peptide, p1091 (SEQ ID NO:20) to two other already known HLA-A2 peptides derived from homologous region in MAGE-A4 GVYDGREHTV (SEQ ID NO:38) [27] and MAGE-A10 proteins GLYDGMEHL (SEQ ID NO:39) [28].

FIG. 5 shows an example of reconstitution of peptides into cells surface MHC to test their binding and affinity as assayed by FACS analysis. Synthetic peptides were added to 10^6 RMA-S-HHD cells to a concentration of 100 μM followed by incubation for two hours at 26°C and two hours at 37°C. The stability of the peptides binding to the HHD cells was measured by indirect FACS assay after decoration for another hour with the W6/32 mAb at 4°C and 30 minutes incubation with FITC goat anti-mouse Ab at 4°C. The HLA-A2.1 peptide derived from gp100 served as a positive control and unloaded RMA-S-HHD cells as a negative control.

FIG. 6 demonstrates a CTL assay with murine cells presenting human MHC (EL4-HHD). Cells were loaded separately with individual peptides,

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washed and injected in four groups: 1- p1028 (SEQ ID NO:13) alone, 2- p1258 (SEQ ID NO:24) alone, 3- pool of peptides: p913 (SEQ ID NO:5), p958 (SEQ ID NO:9), p989 of CD59 (SEQ ID NO:11) and p989 of FLI (SEQ ID NO:12), 4- peptides p1031 (SEQ ID NO:14), p1121 (SEQ ID NO:22) and p1068 (SEQ ID NO:16). Unloaded EL4-HHD or targets cells not loaded with the peptides were used as negative controls. An effector-to-target ratio of 50:1 is shown.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of a method of identifying peptides of a desired origin, such as tumor associated antigens, pathogen (e.g., virus, bacteria) derived antigens, endogenous cytokines, etc., which are capable of binding to MHC molecules of a particular haplotype. The present invention is further of peptides identified by the method and pharmaceutical compositions containing the peptides. Still, the present invention is further of databases describing the peptides and the use of the peptides in vaccination to treat and/or prevent various pathologies, cancer and autoimmune diseases, in particular.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the

details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

According to one aspect of the present invention there is provided a method of identifying peptides originating from a particular cell type and being capable of binding to MHC molecules of a particular haplotype. The method according to this aspect of the present invention is effected by obtaining a cell type expressing a soluble and secreted form of the MHC molecules of the particular haplotype; collecting the soluble and secreted form of the MHC molecules of the particular haplotype; and analyzing peptides bound to the soluble and secreted form of the MHC molecules of the particular haplotype, thereby identifying the peptides originating from the particular cell type and being capable of binding to MHC molecules of the particular haplotype.

According to another aspect of the present invention there is provided a method of identifying peptides originating from cancer associated proteins and being capable of binding to MHC molecules of a particular haplotype. The method according to this aspect of the present invention is effected by obtaining a cancer cell type expressing a soluble and secreted form of the MHC molecules of the particular haplotype; collecting the soluble and secreted form of the MHC molecules of the particular haplotype; analyzing peptides

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bound to the soluble and secreted form of the MHC molecules of the particular haplotype; and identifying peptides originating from cancer associated proteins and being capable of binding to MHC molecules of the particular haplotype.

According to still another aspect of the present invention there is provided a method of identifying peptides originating from cells participating in the development and/or progression of an autoimmune disease and being capable of binding to MHC molecules of a particular haplotype. The method according to this aspect of the present invention is effected by obtaining cells participating in the development and/or progression of the autoimmune disease and expressing a soluble and secreted form of the MHC molecules of the particular haplotype; collecting the soluble and secreted form of the MHC molecules of the particular haplotype; analyzing peptides bound to the soluble and secreted form of the MHC molecules of the particular haplotype; and identifying peptides originating from proteins participating in the development and/or progression of the autoimmune disease and being capable of binding to MHC molecules of the particular haplotype.

According to another aspect of the present invention there is provided a method of identifying peptides originating from virus infected cells and being capable of binding to MHC molecules of a particular haplotype. The method according to this aspect of the present invention is effected by obtaining virus infected cells expressing a soluble and secreted form of the MHC molecules of the particular haplotype; collecting the soluble and secreted form of the MHC

molecules of the particular haplotype; analyzing peptides bound to the soluble and secreted form of the MHC molecules of the particular haplotype; and identifying peptides originating from the virus and being capable of binding to MHC molecules of the particular haplotype.

According to still another aspect of the present invention there is provided a method of identifying peptides originating from a particular cell type characterized by at least one of the following (i) cell over-expressing at least one protein; (ii) cells characterized by induced mutations; (iii) cells of metastases; (iv) normal or transformed cells expressing cell surface proteins, the peptides being capable of binding to MHC molecules of a particular haplotype. The method according to this aspect of the present invention is effected by obtaining cells of the particular cell type expressing a soluble and secreted form of the MHC molecules of the particular haplotype; collecting the soluble and secreted form of the MHC molecules of the particular haplotype; analyzing peptides bound to the soluble and secreted form of the MHC molecules of the particular haplotype; and identifying peptides originating from the particular cell type and being capable of binding to MHC molecules of the particular haplotype.

In general, the present invention provides a method of identifying peptides originating from at least one protein of interest or an unknown protein and being capable of binding to MHC molecules of a particular haplotype. The method is effected by obtaining cells co-expressing the at least one protein

of interest or unknown protein and a soluble and secreted form of the MHC molecules of the particular haplotype; collecting the soluble and secreted form of the MHC molecules of the particular haplotype; analyzing peptides bound to the soluble and secreted form of the MHC molecules of the particular haplotype; and identifying peptides originating from the at least one protein of interest or unknown protein and being capable of binding to MHC molecules of the particular haplotype. Depending to a great extent on the cell type employed, it will Once a peptide of an unknown protein is identified, this protein becomes a protein of interest.

The protein of interest or unknown protein can be a native protein expressed by the cells, or the protein of interest can be expressed by the cells following transformation of the cells with nucleic acid encoding for the protein of interest using techniques well known in the art.

The method of the present invention can thus be used to associate proteins of yet unknown pattern of expression with particular tissues or cell types, such as cancer cells. In addition, the method of the present invention can be used to determine whether a specific open reading frame (ORF) is expressed or not in certain cells.

In one preferred embodiment of the present invention the cell type is a cancer cell or a cancer cell line. Primary cell lines, metastatic cell lines, tumor cell lines and normal cell lines which are suitable for implementing the method

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of the present invention are available, for example, from ATCC. Tables 1 and 2 below provide examples:

TABLE 1
Primary and metastatic cell lines

Primary Cell Line				Metastatic Cell Line		
ATCC No.	Name	Disease	Tissue	ATCC No.	Name	Tissue
CCL-228	SW480	colorectal adenocarcinoma	colon	CCL-227	SW620	lymph node
CRL-1864	RF-1	gastric adenocarcinoma	stomach	CRL-1863	RF-48	ascites
CRL-1675	WM-115	melanoma	skin	CRL-1676	WM-266-4	n/a
CRL-7425	Hs 688(A).T	melanoma	skin	CRL-7426	Hs 688(B).T	lymph node

TABLE 2
Tumor and normal cell lines

Tumor Cell Line				Normal Cell Line		
ATCC No.	Name	Cancer Type	Tissue Source	ATCC No.	Name	Tissue Source
CCL-256	NCI-H2126	carcinoma; non-small cell lung cancer	lung	CCL-256.1	NCI-BL2126	peripheral blood
CRL-5868	NCI-H1395	adenocarcinoma	lung	CRL-5957	NCI-BL1395	peripheral blood
CRL-5872	NCI-H1437	adenocarcinoma	lung	CRL-5958	NCI-BL1437	peripheral blood
CRL-5882	NCI-H1648	adenocarcinoma	lung	CRL-5954	NCI-BL1648	peripheral blood
CRL-5911	NCI-H2009	adenocarcinoma	lung	CRL-5961	NCI-BL2009	peripheral blood
CRL-5985	NCI-H2122	adenocarcinoma	pleural effusion	CRL-5967	NCI-BL2122	peripheral blood
CRL-5922	NCI-H2087	adenocarcinoma	lymph node (metastasis)	CRL-5965	NCI-BL2087	peripheral blood
CRL-5886	NCI-H1672	carcinoma; classic small cell lung cancer	lung	CRL-5959	NCI-BL1672	peripheral blood
CRL-5929	NCI-H2171	carcinoma; small cell lung cancer	lung	CRL-5969	NCI-BL2171	peripheral blood
CRL-5931	NCI-H2195	carcinoma; small cell lung cancer	lung	CRL-5956	NCI-BL2195	peripheral blood
CRL-5858	NCI-H1184	carcinoma; small cell lung cancer	lymph node (metastasis)	CRL-5949	NCI-BL1184	peripheral blood
HTB-172	NCI-H209	carcinoma; small cell lung cancer	bone marrow (metastasis)	CRL-5948	NCI-BL209	peripheral blood
CRL-5983	NCI-H2107	carcinoma, small cell lung cancer	bone marrow (metastasis)	CRL-5966	NCI-BL2107	peripheral blood
HTB-120	NCI-H128	carcinoma; small cell lung cancer	pleural effusion	CRL-5947	NCI-BL128	peripheral blood
CRL-5915	NCI-H2052	mesothelioma	pleural effusion	CRL-5963	NCI-BL2052	peripheral

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						blood
CRL-5893	NCI-H1770	neuroendocrine carcinoma	lymph node (metastasis)	CRL-5960	NCI-BL1770	peripheral blood
HTB-126	Hs 578T	ductal carcinoma	mammary gland; breast	HTB-125	Hs 578Bst	mammary gland; breast
CRL-2320	HCC1008	ductal carcinoma	mammary gland; breast	CRL-2319	HCC1007 BL	peripheral blood
CRL-2338	HCC1954	ductal carcinoma	mammary gland; breast	CRL-2339	HCC1954 BL	peripheral blood
CRL-2314	HCC38	primary ductal carcinoma	mammary gland; breast	CRL-2346	HCC38 BL	peripheral blood
CRL-2321	HCC1143	primary ductal carcinoma	mammary gland; breast	CRL-2362	HCC1143 BL	peripheral blood
CRL-2322	HCC1187	primary ductal carcinoma	mammary gland; breast	CRL-2323	HCC1187 BL	peripheral blood
CRL-2324	HCC1395	primary ductal carcinoma	mammary gland; breast	CRL-2325	HCC1395 BL	peripheral blood
CRL-2331	HCC1599	primary ductal carcinoma	mammary gland, breast	CRL-2332	HCC1599 BL	peripheral blood
CRL-2336	HCC1937	primary ductal carcinoma	mammary gland; breast	CRL-2337	HCC1937 BL	peripheral blood
CRL-2340	HCC2157	primary ductal carcinoma	mammary gland; breast	CRL-2341	HCC2157 BL	peripheral blood
CRL-2343	HCC2218	primary ductal carcinoma	mammary gland; breast	CRL-2363	HCC2218 BL	peripheral blood
CRL-7345	Hs 574.T	ductal carcinoma	mammary gland; breast	CRL-7346	Hs 574.Sk	skin
CRL-7482	Hs 742.T	scirrhous adenocarcinoma	mammary gland; breast	CRL-7481	Hs 742.Sk	skin
CRL-7303	Hs 496.T	cancer	mammary gland; breast	CRL-7302	Hs 496.Sk	skin
CRL-7486	Hs 748.T	cancer	mammary gland, breast	CRL-7485	Hs 748.Sk	skin
CRL-7365	Hs 605.T	carcinoma	mammary gland; breast	CRL-7364	Hs 605.Sk	skin
CRL-7368	Hs 606	carcinoma	mammary gland; breast	CRL-7367	Hs 606.Sk	skin
CRL-1974	COLO 829	malignant melanoma	skin	CRL-1980	COLO 829BL	peripheral blood
CRL-7762	TE 354.T	basal cell carcinoma	skin	CRL-7761	TE 353.Sk	skin
CRL-7690	Hs 939.T	malignant melanoma	skin	CRL-7689	Hs 939 Sk	skin
CRL-7360	Hs 600.T	melanoma	skin	CRL-7359	Hs 600.Sk	skin
CRL-7677	Hs 925.T	pagetoid sarcoma	skin	CRL-7676	Hs 925 Sk	skin
CRL-7672	Hs 919.T	benign osteoid osteoma	bone	CRL-7671	Hs 919.Sk	skin
CRL-7554	Hs 821 T	giant cell sarcoma	bone	CRL-7553	Hs 821.Sk	skin
CRL-7552	Hs 820.T	heterophilic osteofication	bone	CRL-7551	Hs 820.Sk	skin
CRL-7444	Hs 704.T	osteosarcoma	bone	CRL-7443	Hs 704.Sk	skin
CRL-7448	Hs 707(A).T	osteosarcoma	bone	CRL-7449	Hs 707(B).Ep	skin
CRL-7471	Hs 735.T	osteosarcoma	bone	CRL-7865	Hs 735.Sk	skin
CRL-7571	Hs 836.T	osteosarcoma	bone	CRL-7570	Hs 836.Sk	skin
CRL-7595	Hs 860.T	osteosarcoma	bone	CRL-7519	Hs 791.Sk	skin

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CRL-7622	Hs 888.T	osteosarcoma	bone	CCL-211	Hs888Lu	lung
CRL-7626	Hs 889.T	osteosarcoma	bone	CRL-7625	Hs 889.Sk	skin
CRL-7628	Hs 890.T	osteosarcoma	bone	CRL-7627	Hs 890.Sk	skin
CRL-7453	Hs 709.T	periostitis; granuloma	bone	CRL-7452	Hs 709.Sk	skin
CRL-7432	Hs 696.T	adenocarcinoma	unknown	CRL-7431	Hs 696.Sk	skin
CRL-7886	Hs 789.T	transitional cell carcinoma	ureter	CRL-7518	Hs 789.Sk	skin
CRL-7547	Hs 814.T	giant cell sarcoma	vertebral column	CRL-7546	Hs 814.Sk	skin

In another preferred embodiment of the invention, the cell type is a virus infected cell or cell line. Table 3 below provides examples of some known viruses and the diseases they cause:

TABLE 3

Diseases	Viruses and other pathogens
African sleeping sickness (African trypanosomiasis)	Trypanosoma brucei
AIDS	HIV
Amebiasis	Entamoeba histolytica
BSE ("mad cow disease") and nvCJD	
Campylobacter infections	Campylobacter
Chagas' disease (American trypanosomiasis)	Trypanosoma cruzi
Cholera	Vibrio cholerae
Coccidioidomycosis	Coccidioides immitis
Cryptosporidiosis	Cryptosporidium
Cyclosporiasis	Cyclospora
Dengue fever	Dengue viruses
Diphtheria, tetanus, and pertussis	Toxin-producing strains of Corynebacterium diphtheriae
Bordetella pertussis	
Encephalitis	Japanese encephalitis virus Tickborne encephalitis West Nile virus
Filariasis	Wuchereria bancrofti and Brugia malayi
Giardiasis (Giardia infection)	Giardia intestinalis
Hantavirus pulmonary syndrome	Hantavirus
Hepatitis	Hepatitis viruses A, B, C, E
Histoplasmosis	Histoplasma capsulatum
Influenza (flu)	
Leishmaniasis	Leishmania
Leptospirosis	Leptospira
Lyme disease	B. burgdorferi sensu stricto, B. afzelii, or B. garinii
Malaria	Plasmodium falciparum P. vivax P. ovale and P. malariae
Measles, mumps, and rubella (MMR)	
Meningitis	Haemophilus influenzae type b Streptococcus pneumoniae and Neisseria meningitidis
Onchocerciasis (river blindness)	Onchocerca volvulus
Plague	Yersinia pestis

Poliomyelitis	
Rabies	Rhabdoviridae, genus Lyssavirus
Rocky Mountain spotted fever	
Rickettsia	rickettsii
severe diarrhea	Rotavirus
Salmonellosis	Salmonella
Schistosomiasis	
Shigellosis	Shigella
Tuberculosis (TB)	Mycobacterium tuberculosis
Typhoid fever	Salmonella serogroup Typhi
Typhus fevers	rickettsiae
chickenpox	Varicella
Vibrio parahaemolyticus	
Viral hemorrhagic fevers (e.g., Ebola, Lassa, Marburg, Rift Valley).	arenaviruses, filoviruses, bunyaviruses, and flaviviruses
Yellow fever	

In yet another preferred embodiment of the present invention, the cell type is a cell involved in a development and/or progression of an autoimmune diseases such as T or B cells, and/or an allergic disease or condition, such as mast cells.

In one example, the at least one protein of interest is a tumor associated antigen. The tumor associated antigen can be natively expressed by the cells or can be expressed by appropriately transformed cells. Table 4 below lists some known genes encoding proteins which were identified as tumor associated antigens.

TABLE 4

Gene Symbol	Gene Name	Locus	Disorders
ABL1	v-abl Abelson murine leukemia viral oncogene homolog 1	9q34.1	Leukemia, chronic myeloid
ABL2	v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene)	1q24-q25	Leukemia, acute myeloid, with eosinophilia
AKT2	v-akt murine thymoma viral oncogene homolog 2	19q13 1-q13.2	Ovarian carcinoma
ARHI	ras homolog gene family, member I	1p31	Ovarian cancer
ARP		3p21.1	Pancreatic cancer
AXIN2	axin 2 (conductin, axil)	17q23-q24	Colorectal cancer

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BAX	BCL2-associated X protein	19q13.3-q13.4	Colorectal cancer T-cell acute lymphoblastic leukemia
BCPR	homeo box B9	17p13 3	Breast cancer
BRCA1	breast cancer 1, early onset	17q21	Breast cancer-1 Ovarian cancer Breast-ovarian cancer
BRCA2	breast cancer 2, early onset	13q12.3	Breast cancer 2, early onset Pancreatic cancer
BRCA3		11q23	Breast cancer-3
BRCA4		13q21	Breast cancer, type 4
BRCAX		13q21	Breast cancer, type 4
BRCD1		13	Breast cancer, ductal
BRCD2		1p36	Breast cancer, ductal
BUB1	budding uninhibited by benzimidazoles 1 (yeast homolog)	2q14	Colorectal cancer with chromosomal instability
CDH1	cadherin 1, type 1, E-cadherin (epithelial)	16q22 1	Endometrial carcinoma Ovarian carcinoma Breast cancer,
CLD	congenital chloride diarrhea	7q22-q31 1	Colon cancer Chloride diarrhea, congenital, Finnish type,
CSF1R	colony stimulating factor 1 receptor, formerly McDonough feline sarcoma viral (v-fms) oncogene homolog	5q33.2-q33 3	Myeloid malignancy, predisposition to
CTNNB1	catenin (cadherin-associated protein), beta 1 (88kD)	3p22-p21 3	Colorectal cancer Hepatoblastoma Pilomatricoma
CYLD	cylindromatosis (turban tumor syndrome)	16q12-q13	Cylindromatosis, familial
DCC	deleted in colorectal carcinoma	18q21.3	Colorectal cancer
DEK	DEK oncogene (DNA binding)	6p23	Leukemia, acute nonlymphocytic
DLEC1	deleted in lung and esophageal cancer 1	3p22-p21.3	Lung cancer Esophageal cancer
DMBT1	deleted in malignant brain tumors 1	10q25.3-q26.1	Glioblastoma multiforme Medulloblastoma
DRA	down-regulated in adenoma	7q22-q31.1	Colon cancer Chloride diarrhea, congenital, Finnish type,
ELAC2	elaC (E coli) homolog 2	17p	Prostate cancer, susceptibility to
EP300	E1A binding protein p300	22q13	Colorectal cancer
ESR1	estrogen receptor 1	6q25.1	Breast cancer Estrogen resistance
ETV6	ets variant gene 6 (TEL oncogene)	12p13	Leukemia, acute lymphoblastic
FSHR	follicle stimulating hormone receptor	2p21-p16	Premature ovarian failure Ovarian sex cord tumors
HNPCC7	3346	15q21.1	Colorectal cancer, hereditary nonpolyposis, type 7
HPC1	hereditary prostate cancer 1	1q24-q25	Prostate cancer, susceptibility to
HPCX	hereditary prostate cancer, X-linked	Xq27-q28	Prostate cancer, susceptibility to
HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	11p15 5	Bladder cancer
HRPT2	hyperparathyroidism 2 (with jaw tumor)	1q25-q31	Hyperparathyroidism-jaw tumor syndrome Hyperparathyroidism,
KAI1	kangai 1 (suppression of tumorigenicity 6, prostate; CD82 antigen (R2 leukocyte antigen, antigen detected by monoclonal and antibody IA4))	11p11.2	Prostate cancer, susceptibility to

KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	4q12	Piebaldism Mast cell leukemia Mastocytosis with associated
KRAS1P	v-Ki-ras1 Kirsten rat sarcoma 1 viral oncogene homolog, processed pseudogene	12p12.1	Colorectal adenoma Colorectal cancer
KRAS2	v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	12p12.1	Colorectal adenoma Colorectal cancer
LCFS2	mitochondrial ribosomal protein L13	18q11-q12	?Lynch cancer family syndrome II
LCO	liver cancer oncogene	2q14-q21	Hepatocellular carcinoma
MADH4	MAD (mothers against decapentaplegic, Drosophila) homolog 4	18q21 1	Pancreatic cancer Polyposis, juvenile intestinal
MCC	mutated in colorectal cancers	5q21	Colorectal cancer
MERTK	c-mer proto-oncogene tyrosine kinase	2q14.1	Retinitis pigmentosa, MERTK-related
MET	met proto-oncogene (hepatocyte growth factor receptor)	7q31	Renal cell carcinoma, papillary, familial and sporadic
MGCT		12q22	Male germ cell tumor
MLH1	mutL (E. coli) homolog 1 (colon cancer, nonpolyposis type 2)	3p21 3	Colorectal cancer, hereditary nonpolyposis, type 2
MPL	myeloproliferative leukemia virus oncogene	1p34	Thrombocytopenia, congenital amegakaryocytic
MSH2	mutS (E. coli) homolog 2 (colon cancer, nonpolyposis type 1)	2p22-p21	Colorectal cancer, hereditary nonpolyposis, type 1
MSH6	mutS (E coli) homolog 6	2p16	Cancer susceptibility Endometrial carcinoma Colorectal
MTACR1	multiple tumor-associated chromosome region 1	11p15.5	Wilms tumor, type 2 Adrenocortical carcinoma, hereditary, 202300
MYC	v-myc avian myelocytomatosis viral oncogene homolog	8q24.12-q24.13	Burkitt lymphoma
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog	1p13 2	Colorectal cancer
PCAP	predisposing for prostate cancer	1q42.2-q43	Prostate cancer, susceptibility to
PCBC	3475	1p36	Prostate cancer, susceptibility to
PDGFB	platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)	22q12.3-q13.1	Meningioma, SIS-related Dermatofibrosarcoma protuberans
PDGFRL	platelet-derived growth factor receptor-like	8p22-p21 3	Hepatocellular cancer Colorectal cancer
PGL2	paraganglioma or familial glomus tumors 2	11q13.1	Paraganglioma, familial nonchromaffin
PGL3	paraganglioma or familial glomus tumors 3	1q21	∴Paragangliomas, familial nonchromaffin, 3
PHB	prohibitin	17q21	Breast cancer, sporadic
PIK3CA	phosphoinositide-3-kinase, catalytic, alpha polypeptide	3q26 3	Ovarian cancer
PMS1	postmeiotic segregation increased (S. cerevisiae) 1	2q31-q33	Colorectal cancer, hereditary nonpolyposis, type 3
PMS2	postmeiotic segregation increased (S. cerevisiae) 2	7p22	Turcot syndrome with glioblastoma Colorectal cancer,
PPP2R1B	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), beta isoform	11q22-q24	Lung cancer
PRCA1	prostate cancer 1	1q24-q25	Prostate cancer, susceptibility to
PRKCA	protein kinase C, alpha	17q22-q23 2	Pituitary tumor, invasive
PTEN	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	10q23.3	Cowden disease Lhermitte-Duclos syndrome
PTPN12	protein tyrosine phosphatase, non-receptor type 12	7q11.23	Colon cancer

RAB27A	RAB27A, member RAS oncogene family	15q21	Griscelli syndrome
RAD51	RAD51 (S cerevisiae) homolog (E coli RecA homolog)	15q15 1	Breast cancer, susceptibility to
RAD54L	RAD54 (S.cerevisiae)-like	1p32	Lymphoma, non-Hodgkin Breast cancer, invasive intraductal
RB1	retinoblastoma 1 (including osteosarcoma)	13q14.1-q14 2	Retinoblastoma Osteosarcoma Bladder cancer,
RET	ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)	10q11 2	Multiple endocrine neoplasia IIA Medullary thyroid
RUNX1	runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)	21q22 3	Leukemia, acute myeloid Platelet disorder, familial, with
SCLC1	354	3p23-p21	Small-cell cancer of lung
SLC22A1L	solute carrier family 22 (organic cation transporter), member 1-like	11p15 5	Breast cancer Rhabdomyosarcoma Lung
SLC26A3	solute carrier family 26 , member 3	7q22-q31.1	Colon cancer Chloride diarrhea, congenital, Finnish type
SMARCB1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	22q11	Rhabdoid tumors Rhabdoid predisposition syndrome, familial
SRC	v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog	20q12-q13	Colon cancer, advanced
SSTR2	somatostatin receptor 2	17q24	Lung cancer, small cell
ST11	suppression of tumorigenicity 11 (pancreas)	3p25	Pancreatic endocrine tumors
ST12	suppression of tumorigenicity 12 (prostate)	10pter-q11	Prostate adenocarcinoma
ST3	suppression of tumorigenicity 3	11q13	Cervical carcinoma
ST8	suppression of tumorigenicity 8 (ovarian)	6q26-q27	Ovarian cancer, serous
TACSTD2	tumor-associated calcium signal transducer 2	1p32-q12	Corneal dystrophy, gelatinous drop-like
TCF7L2	transcription factor 7-like 2 (T-cell specific, HMG-box)	10q25.3	Colorectal cancer
TGFBR2	transforming growth factor, beta receptor II (70-80kD)	3p22	Colon cancer Colorectal cancer, hereditary nonpolyposis, type 6
THPO	thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte growth and development factor)	3q26.3-q27	Thrombocythemia, essential
TNFRSF10B	tumor necrosis factor receptor superfamily, member 10b	8p22-p21	Squamous cell carcinoma, head and neck
TNFRSF11A	tumor necrosis factor receptor superfamily, member 11a, activator of NFKB	18q22.1	Osteolysis, familial expansile Paget disease of bone,
TNFRSF1A	tumor necrosis factor receptor superfamily, member 1A	12p13.2	Periodic fever, familial
TNFRSF6	tumor necrosis factor receptor superfamily, member 6	10q24 1	Autoimmune lymphoproliferative syndrome
TNFSF5	tumor necrosis factor (ligand) superfamily, member 5 (hyper-IgM syndrome)	Xq26	Immunodeficiency, X-linked, with hyper-IgM
TNFSF6	tumor necrosis factor (ligand) superfamily, member 6	1q23	Systemic lupus erythematosus, susceptibility to
TNF	tumor necrosis factor (TNF superfamily, member 2)	6p21.3	Malaria, cerebral, susceptibility to Septic shock
TOC	tylosis with oesophageal cancer	17q24	Tylosis with esophageal cancer
TP53	tumor protein p53 (Li-Fraumeni syndrome)	17p13.1	Colorectal cancer Li-Fraumeni syndrome

TP73	tumor protein p73	1p36	Neuroblastoma
TSG101	tumor susceptibility gene 101	11p15.2-p15.1	Breast cancer
VMGLOM	venous malformation with glomus cells	1p22-p21	Glomus tumors, multiple
WT1	Wilms tumor 1	11p13	Wilms tumor, type 1 Denys-Drash syndrome Frasier
WT2	Wilms tumor 2	11p15.5	Wilms tumor, type 2 Adrenocortical carcinoma, hereditary

In another example, the at least one protein of interest includes a cytokine. Many diseases, including neurodegenerative (e.g., Alzheimer's disease) and autoimmune (e.g., rheumatoid arthritis, multiple sclerosis and the like) diseases are caused or accompanied by inflammation, resulting in infiltration of leukocytes into the inflicted tissue(s). In these diseases proinflammatory cytokines and chemokines are believed to play a pivotal role in the attraction of leukocytes to the site of inflammation and in the initiation and progression of the inflammatory process. In rheumatoid arthritis, for example, the role of proinflammatory cytokines, particularly TNF-o and IL-1, in disease manifestation has been intensively studied and explored in experimental models that have been expanded to clinical trials. Other cytokines such as IL-4, TGF-β, IL-8, IL-17, IL-10, IL-11, IL-12 and IL-15 have also been implicated in the regulation of rheumatoid arthritis. Such regulation can be attributed to either their direct effect on disease manifestation, their synergistic effect with other proinflammatory cytokines/chemokines, or their involvement in the regulation of chemokine transcription, and production.

Chemokines are chemoattractant cytokines that mediate leukocyte attraction and recruitment at the site of inflammation. Based on the positions

of the first two cysteines, chemokines can be divided into four highly conserved but distinct supergene families, C-C, C-X-C, C and C-X3-C. The C-C family is primarily involved in the activation of endothelium and chemoattraction of T cells and monocytes to the site of inflammation. The protective competence of anti-C-C chemokine based immunotherapy has been demonstrated in experimental autoimmune encephalomyelitis (EAE), and rheumatoid arthritis.

Neutralizing the activity of chemokines as a way to treat various diseases has been explored by many researchers. For example, in a recent study neutralizing antibodies to epithelial neutrophil activating peptide 78 (ENA-78) were found capable of inhibiting the development of AA if administered before but not after the onset of disease [92]. In another recent study, Barnes *et al.* [93] used anti-human RANTES to ameliorate AA in the Lewis rat. Gong *et al.* [94] used an antagonist of Monocyte Chemoattractant Protein 1 (MCP-1) to inhibit arthritis in the MRL-lpr mouse model. Using a streptococcal cell wall induced arthritis model it has been shown that anti-IL-4 and anti MCP-1 antibodies block the disease [95]. The same study demonstrated that neutralizing IL-4 by itself, leads to a marked reduction in MCP-1 mRNA transcription at the autoimmune site and to inhibition of the development of disease which further implicates MCP-1 in playing an active role in arthritis development.

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In yet another example, the at least one protein of interest includes a protein, e.g., a surface protein, of a pathogen, such as a viral pathogen, a bacterial pathogen or a parasite (either mono or multicellular parasite).

The major histocompatibility complex (MHC) is a complex of antigens encoded by a group of linked loci, which are collectively termed H-2 in the mouse and HLA in humans. The two principal classes of the MHC antigens, class I and class II, each comprise a set of cell surface glycoproteins which play a role in determining tissue type and transplant compatibility. In transplantation reactions, cytotoxic T-cells (CTLs) respond mainly against foreign class I glycoproteins, while helper T-cells respond mainly against foreign class II glycoproteins.

Major histocompatibility complex (MHC) class I molecules are expressed on the surface of nearly all cells. These molecules function in presenting peptides which are mainly derived from endogenously synthesized proteins to CD8+ T cells via an interaction with the αβ T-cell receptor. The class I MHC molecule is a heterodimer composed of a 46-kDa heavy chain which is non-covalently associated with the 12-kDa light chain β-2 microglobulin. Class I MHC-restricted peptides, which are traditionally assumed to be 8-10-amino acid-long, bind to the heavy chain α1-α2 groove via two or three anchor residues that interact with corresponding binding pockets in the MHC molecule. The β-2 microglobulin chain plays an important role in MHC class I intracellular transport, peptide binding, and conformational

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stability [76]. For most class I molecules, the formation of a heterodimer consisting of the MHC class I heavy chain, peptide (self or antigenic) and β-2 microglobulin is required for biosynthetic maturation and cell-surface expression [76].

Research studies performed on peptide binding to class I MHC molecules enable to define specific MHC motifs functional in displaying peptides derived from viral or tumor antigens that are potentially immunogenic and might elicit specific response from cytotoxic T lymphocytes (CTLs) [77,78].

Soluble MHC multimers posses a high avidity for T-cells since they provide multi-point binding of TCRs with their MHC-peptide ligands. As such, multimeric forms (tetramers) of MHC-peptide complexes have been the center of much interest recently, because they can be used for direct phenotypic characterization of T cell responses in normal as well as pathological conditions, thus, providing insight into the pathopysiology and mechanisms of various diseases. Recombinant soluble and secreted MHC class I and class II complexes including single chain MHC are described in [79-91] which are incorporated herein by references.

There are several thousands of MHC genes, some of which were cloned. Table 5 below associates the MHC genes into classes and types (6). The sequences of the known MHC genes can be found in the Kabat database (http://immuno.bme.nwu.edu/).

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TABLE 5

	Type	Number of genes
MHC Class I	A, B, C	1014
MHC class II A chain	DR DQ DP	348
MHC class II B chain	DR DQ DP	1680

Genes encoding MHC of particular haplotypes can be readily isolated using techniques well known in the art and reconstructed to encode soluble MHC molecules essentially as exemplified in the Examples section that follows. Such well known techniques include, for example, PCR amplification, enzymatic digestion and ligation.

According to a presently preferred embodiment of the present invention analyzing the peptides bound to the soluble and secreted form of the MHC molecules of the particular haplotype is by mass spectrometry, mass charge ratio and collision induced disintegration. Edman degradation can also be employed in certain cases where a sufficient amount of the pure peptide becomes available.

The identification of the amino acid sequence of a peptide in accordance with the teachings of the present invention is preferably effected by comparison of the data collected by mass spectrometry, mass charge ratio and collision induced disintegration to putative data of mass spectrometry, mass charge ratio and collision induced disintegration of known proteins.

As used herein in the specification and in the claims section below the term "peptide" includes native peptides (either degradation products or synthetically synthesized peptides) and further to peptidomimetics, such as

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peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body, or more immunogenic.

Such modifications include, but are not limited to, cyclization, N terminus modification, C terminus modification, peptide bond modification, including, but not limited to, CH_2-NH, CH_2-S, CH_2-S=O, O=C-NH, CH_2-O, CH_2-CH_2, S=C-NH, CH=CH or CF=CH, backbone modification and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further detail in this respect are provided hereinunder.

As used herein in the specification and in the claims section below the term "amino acid" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally *in vivo*, including for example hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids. Further elaboration of the possible amino acids usable according to the present invention and examples of non-natural amino acids useful in MHC class I, type

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A2, recognizable peptide antigens are given hereinunder. Other anchor residues are known for other MHC molecules.

Thus, assume the following positions (P1-P9) in a 9-mer peptide:

P1-P2-P3-P4-P5-P6-P7-P8-P9

The P2 and P9 positions include the anchor residues which are the main residues participating in binding to A2 MHC molecules. Amino acid resides engaging positions P2 and P9 are hydrophilic aliphatic non-charged natural amino (examples being Ala, Val, Leu, Ile, Gln, Thr, Ser, Cys, preferably Val and Leu) or of a non-natural hydrophilic aliphatic non-charged amino acid (examples being norleucine (Nle), norvaline (Nva), α-aminobutyric acid). Positions P1 and P3 are also known to include amino acid residues which participate or assist in binding to MHC molecules, however, these positions can include any amino acids, natural or non-natural. The other positions are engaged by amino acid residues which typically do not participate in binding, rather these amino acids are presented to the immune cells. Further details relating to the binding of peptides to MHC molecules can be found in reference 117, see Table V thereof, in particular.

Hydrophilic aliphatic natural amino acids at P2 and P9 can be substituted by synthetic amino acids, preferably Nleu, Nval and/or α -aminobutyric acid. P9 can be also substituted by aliphatic amino acids of the general formula $-HN(CH_2)_nCOOH$, wherein n = 3-5, as well as by branched derivatives thereof, such as, but not limited to,

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$$-NH(CH_2)_n\text{-}COON$$
$$|$$
$$R$$

wherein R is, for example, methyl, ethyl or propyl, located at any one or more of the n carbons.

The amino terminal residue (position P1) can be substituted by positively charged aliphatic carboxylic acids, such as, but not limited to, $H_2N(CH_2)_nCOOH$, wherein n = 2-4 and $H_2N\text{-}C(NH)\text{-}NH(CH_2)_nCOOH$, wherein n = 2-3, as well as by hydroxy Lysine, N-methyl Lysine or ornithine (Orn). Additionally, the amino terminal residue can be substituted by enlarged aromatic residues, such as, but not limited to, $H_2N\text{-}(C_6H_6)\text{-}CH_2\text{-}COOH$, p-aminophenyl alanine, $H_2N\text{-}F(NH)\text{-}NH\text{-}(C_6H_6)\text{-}CH_2\text{-}COOH$, p-guanidinophenyl alanine or pyridinoalanine (Pal). These latter residues may form hydrogen bonding with the OH^- moieties of the Tyrosine residues at the MHC-1 N-terminal binding pocket, as well as to create, at the same time aromatic-aromatic interactions.

Derivatization of amino acid residues at positions P4-P8, should these residues have a side-chain, such as, OH, SH or NH_2, like Ser, Tyr, Lys, Cys or Orn, can be by alkyl, aryl, alkanoyl or aroyl. In addition, OH groups at these positions may also be derivatized by phosphorylation and/or glycosylation. These derivatizations have been shown in some cases to enhance the binding to the T cell receptor.

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Longer derivatives in which the second anchor amino acid is at position P10 may include at P9 most L amino acids. In some cases shorter derivatives are also applicable, in which the C terminal acid serves as the second anchor residue.

Cyclic amino acid derivatives can engage position P4-P8, preferably positions P6 and P7. Cyclization can be obtained through amide bond formation, e.g., by incorporating Glu, Asp, Lys, Orn, di-amino butyric (Dab) acid, di-aminopropionic (Dap) acid at various positions in the chain (-CO-NH or -NH-CO bonds). Backbone to backbone cyclization can also be obtained through incorporation of modified amino acids of the formulas H-N((CH_2)$_n$-COOH)-C(R)H-COOH or H-N((CH_2)$_n$-COOH)-C(R)H-NH_2, wherein n = 1-4, and further wherein R is any natural or non-natural side chain of an amino acid. As stated above, the data presented herein relates to the residues of the most abandoned MHC molecule - MHC class I, type A2. This data was collected over the years via the detailed analysis of thousands of peptides that bind to MHC-I, A2. It will be appreciated that the method of the present invention allows the collection of data and analysis of peptides that bind any other to MHC molecule.

Cyclization via formation of S-S bonds through incorporation of two Cys residues is also possible. Additional side-chain to side chain cyclization can be obtained via formation of an interaction bond of the formula -(-CH_2-)$_n$-S-CH_2-C-, wherein n = 1 or 2, which is possible, for example,

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through incorporation of Cys or homoCys and reaction of its free SH group with, e.g., bromoacetylated Lys, Orn, Dab or Dap.

Peptide bonds (-CO-NH-) within the peptide may be substituted by N-methylated bonds (-N(CH_3)-CO-), ester bonds (-C(R)H-C-O-O-C(R)-N-), ketomethylen bonds (-CO-CH_2-), α-aza bonds (-NH-N(R)-CO-), wherein R is any alkyl, e.g., methyl, carba bonds (-CH_2-NH-), hydroxyethylene bonds (-CH(OH)-CH_2-), thioamide bonds (-CS-NH-), olefinic double bonds (-CH=CH-), retro amide bonds (-NH-CO-), peptide derivatives (-N(R)-CH_2-CO-), wherein R is the "normal" side chain, naturally presented on the carbon atom.

These modifications can occur at any of the bonds along the peptide chain and even at several (2-3) at the same time. Preferably, but not in all cases necessary, these modifications should exclude anchor amino acids.

Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted for synthetic non-natural acid such as TIC, naphthylelanine (Nol), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr.

Tables 6-7 below list all of the naturally occurring amino acids (Table 6) and some of the non-conventional or modified amino acids (Table 7).

TABLE 6

Amino Acid	Three-Letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G

Histidine	His	H
Isoleucine	Iie	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any amino acid as above	Xaa	X

Table 7

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-	Cpro	L-N-methylasparagine	Nmasn
carboxylate		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-	Norb	L-N-methylglutamine	Nmgin
carboxylate		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcyclopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn

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D-α-methylisoleucine	Dmile	N- amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cyclododeclglycine	Ncdod
D-α-methylalnine	Dnmala	N-cyclooctylglycine	Ncoct
D-α-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-α-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-α-methylasparatate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-α-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl) glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nva
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(p-hydroxyphenyl)glycine	Nhtyr
L-t-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-t-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomo phenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe

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N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(p-hydroxyphenyl)glycine	Nhtyr
L-t-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-t-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	mser	L-α-methylthreonine	Mthr
L-α-methylvaline	Mtrp	L-α-methyltyrosine	Mtyr
L-α-methylleucine	Mval Nnbhm	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl-glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl(1)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl ethylamino)cyclopropane	Nmbc		

A peptide according to the present invention can be used in a self standing form or be a part of a larger moiety such as a protein or a display moieties such as a display bacterium, a display phage or preferably a display cell.

Additionally, a peptide according to the present invention includes at least five, optionally at least six, optionally at least seven, optionally at least eight, optionally at least nine, optionally at least ten, optionally at least eleven, optionally at least twelve, optionally at least thirteen, optionally at least fourteen, optionally at least fifteen, optionally at least sixteen or optionally at

least seventeen, optionally between seventeen and twenty five or optionally between twenty five and at least thirty amino acid residues (also referred to herein interchangeably as amino acids).

Accordingly, as used herein the term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally *in vivo*, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids.

As used herein the phrase "derived from a protein" refers to peptides derived from the specified protein or proteins and further to homologous peptides derived from equivalent regions of proteins homologous to the specified proteins of the same or other species, provided that these peptides are effective as vaccines, such as anti-tumor vaccines. The term further relates to permissible amino acid alterations and peptidomimetics designed based on the amino acid sequence of the specified proteins or their homologous proteins.

As used herein the phrase "anti-tumor vaccines" refers to a vaccines effective in preventing the development of, or curing, cancer, including primary tumor and/or metastases.

The peptides of the invention can be administered *per se* or as an active ingredient in a pharmaceutical composition which may further include a

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pharmaceutically acceptable carrier. Preferably, one or more peptides of the invention are presented in context of an antigen presenting cell. The most common cells used to load antigens are bone marrow and peripheral blood derived dendritic cells (DC), as these cells express costimulatory molecules that help activation of CTL. Nevertheless, the peptide presenting cell can also be a macrophage, a B cell or a fibroblast. Presenting the peptide can be effected by a variety of methods, such as, but not limited to, (a) transforming the presenting cell with at least one polynucleotide (e.g., DNA) encoding at least one peptide; (b) loading the presenting cell with at least one polynucleotide (e.g., RNA) encoding at least one peptide; (c) loading the presenting cell with at least one peptide (e.g., synthetic peptide); and (d) loading the antigen presenting cell with at least one longer polypeptide (e.g., purified) including at least one peptide. Loading can be external or internal. The polynucleotide, peptide or longer polypeptide can be fused to internalizing sequences, antennapedia sequences or toxoid sequences or to helper sequences, such as, but not limited to, heat shock protein sequences.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the peptides described herein, with other chemical components such as pharmaceutically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to a subject.

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Hereinafter, the term "pharmaceutically acceptable carrier" refers to a carrier or a diluent that does not cause significant irritation to a subject and does not abrogate the biological activity and properties of the administered compound. Examples, without limitations, of carriers are propylene glycol, saline, emulsions and mixtures of organic solvents with water.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

According to a preferred embodiment of the present invention, the pharmaceutical carrier is an aqueous solution of lactic acid.

In this respect, it should be pointed out that some of the peptides of the present invention, according to preferred embodiments, are readily soluble in aqueous media and are therefore easily formulated.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, transdermal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as

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intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more pharmaceutically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the peptides of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer with or without organic solvents such as propylene glycol, polyethylene glycol and the like. For transmucosal administration, penetrants are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the peptides can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the peptides of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries,

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suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores.

Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules

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may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as aqueous solution, fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the peptides are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The peptides described herein may be formulated for parenteral administration, e.g., by bolus injection or continuos infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions

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may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active compound in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the peptides to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The peptides of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

The pharmaceutical compositions herein described may also comprise suitable solid of gel phase carriers or excipients. Examples of such carriers or

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excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin and polymers such as polyethylene glycols.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of peptide effective to prevent, alleviate or ameliorate symptoms of pathology or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any peptide used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from activity assays in cell cultures and/or animals. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC50 as determined by activity assays (e.g., the concentration of the test compound, which achieves a half-maximal inhibition of the proliferation activity). Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the peptides described herein can be determined by standard pharmaceutical procedures in experimental animals, e.g., by determining the IC50 and the LD50 (lethal dose causing death in 50 %

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of the tested animals) for a subject compound. The data obtained from these activity assays and animal studies can be used in formulating a range of dosage for use in human.

The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain therapeutic effects, termed the minimal effective concentration (MEC). The MEC will vary for each preparation, but can be estimated from *in vitro* and/or *in vivo* data, e.g., the concentration necessary to achieve 50-90 % inhibition of a proliferation of certain cells may be ascertained using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using the MEC value. Preparations should be administered using a regimen, which maintains plasma levels above the MEC for 10-90 % of the time, preferable between 30-90 % and most preferably 50-90 %.

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of the tested animals) for a subject compound. The data obtained from these activity assays and animal studies can be used in formulating a range of dosage for use in human.

The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain therapeutic effects, termed the minimal effective concentration (MEC). The MEC will vary for each preparation, but can be estimated from *in vitro* and/or *in vivo* data, e.g., the concentration necessary to achieve 50-90 % inhibition of a proliferation of certain cells may be ascertained using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using the MEC value. Preparations should be administered using a regimen, which maintains plasma levels above the MEC for 10-90 % of the time, preferable between 30-90 % and most preferably 50-90 %.

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Depending on the severity and responsiveness of the condition to be treated, dosing can also be a single administration of a slow release composition described hereinabove, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as a U.S. Food and Drug Administration (FDA) approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a chemical conjugate of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an

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appropriate container, and labeled for treatment of an indicated condition, such as a cancer of a certain type, an autoimmune disease or an allergy.

Peptides of the present invention may be packaged in kits, each such kit comprising a plurality of individual containers, each of which containing at least one peptide identified by the method of the present invention. Such a kit can be used for two purposes. First, an *in vitro* functional assay, such as the CTL assay [20] or the ELISPOT assay [19] of, for example, cytokine (e.g., IL-2, TNF alpha or interferon gamma) production or development of cytotoxiciy using immune cells derived from a patient can be used to determine the immune response of the patient to each one of the peptides, which response to a large extent depends on the particular MHC haplotype of the patient. Second, once, a reactive peptide or peptides are identified, either by an individualized *in vitro* assay or from *in silico* data as is further detailed below, suitable peptide or peptides from the kit can be administered so as to treat the patient.

According to another aspect of the present invention there is provided an electronic data storage device, storing, in a retrievable form, a plurality of sequences of peptides identified by the method described herein. Various other parameters, such as the parameters identified in the Tables provided in the Examples section that follows, can also be linked to the peptide sequences, in, for example, a table form or any other form. Preferably, the plurality of peptides are arranged at least according to their association with a pathology

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and further according to their ability of binding to MHC molecules of a particular haplotype. This *in silico* data can be used instead or in addition to the in vitro assays described above to match a most active peptide to treat a pathology of a certain patient having a particular pre identified MHC haplotype. Thus, look up tables associating a peptide with a protein with a gene, with a disease with a haplotype, and/or with an efficiency score can be constructed and used to best suit a peptide for treatment of a disease in an individualized way taking into account the MHC haplotype of the patient to be treated. Of course, individualized *in vitro* assays can be used to ascertain peptide selection.

The electronic data storage device can, for example, be an electromagnetically or electro-optically readable device and it preferably forms a part of a server that is accessible by users through a communications network, such as the Internet, an intranet or an extranet, via a plurality of user clients at the disposal of the users. A management software application manages the data stored in the data storage device and is preferably designed to support search and retrieval of information from the database and deposition of information into the database.

Thus, further according to the present invention there is provided a method of eliciting an immune response against a protein of interest in a subject having a particular MHC haplotype. The method according to this aspect of the invention is effected by determining the subject's particular MHC

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haplotype; and administering to the subject an effective amount of at least one peptide derived from the protein of interest and which is capable of binding to MHC molecules of the particular haplotype.

Still further according to the present invention there is provided a method of eliciting an immune response against a protein of interest in a subject. The method is effected by using an individualized in vitro assay for determining an immune reactivity of an immune system of the subject to a plurality of peptides derived from the protein of interest; and administering to the subject an effective amount of at least one peptide derived from the protein of interest and which is capable of inducing predetermined sufficient immune reactivity.

According to another aspect the present invention provides a method of treating a pathology by eliciting an immune response against a protein of interest in a subject having a particular MHC haplotype. The method is effected by determining the subject's particular MHC haplotype; and administering to the subject a therapeutic effective amount of at least one peptide derived from the protein of interest and which is capable of binding to MHC molecules of the particular haplotype.

As used herein the term "treating" includes prevention or cure of a pathology, such as a disease, syndrom or manifestation, effected by inhibiting, slowing or reversing the progression of the disease, syndrom or manifestation, substantially ameliorating clinical symptoms of a disease, syndrom or

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manifestation or substantially preventing the appearance of clinical symptoms of a disease, syndrom or manifestation.

As used herein the term "subject" refers to humans and animals having an MHC system, such as the HLA system in humans, in particular farm animals. It will be appreciated in this respect that the method of the present invention can be used to improve all kinds of peptide immunization via individualization for both human beings and animals.

A variety of pathologies can be treated using the peptides of the present invention, including, but not limited to, cancers, infections, inflammations, autoimmune diseases, allergies, etc. The gist of the present invention with respect to treating pathologies lies in the fact that the present invention offers, for the first time, individualization of the vaccine to the MHC haplotype of the treated subject.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

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Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517;

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3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

MATERIALS AND EXPERIMENTAL PROCEDURES

Cell lines:

The human cancer cell lines: PC3 (prostate cancer), UCI-107 and UCI-101 (both ovarian cancer), MDA-231 and MCF-7 (both breast cancer) were obtained from the ATCC. The human B-cell line C1R was a generous gift from Nick Zavazava. UCI-107, UCI-101, MDA-231 and MCF-7 cells were maintained in DMEM containing 10 % FCS, 1 mM glutamine, 0.1 mg/ml

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streptomycin and 100 units/ml penicillin. PC3 and C1R cells were maintained in RPMI 1640 containing 10 % FCS, 1 mM glutamine, 0.1 mg/ml streptomycin and 100 units/ml penicillin. For growing MCF-7 cells without estrogen, the cells were maintained in DMEM without sodium pyruvate and phenol red and containing 4 % FCS stripped of estrogen, 1 mM glutamine, 0.1 mg/ml streptomycin and 100 units/ml penicillin. Culture media, and serum were obtained from GibcoBRL.

RMA-S-HHD is a murine TAP-2 deficient lymphoma clone of C57BL/6 origin, transfected with HLA-A2.1/Db-β2m single chain (HHD) [23]. The RMA-S-HHD-B7.1 cells transfected by the murine B7.1 costimulatory molecule (CD80). EL4-HHD is the murine lymphoma cell line EL4 transfected by HHD. RMA-S-HHD, RMA-S-HHD-B71 and EL4-HHD were maintained in RPMI 1640 containing 10 % FCS and 1 mM glutamine, 0.1 mg/ml streptomycin and 100 units/ml penicillin. After transfection, the cells were maintained in medium supplemented with 500 to 1000 μg/ml of the antibiotic G418 (GibcoBRL).

DNA:

Plasmid HLA-A2/Q10^b, used for expression of soluble MHC, contains the first five exons of the HLA-A2 fused to exons 5 to 8 of the murine mutant Q10^b, which lacks a functional transmembrane domain and is therefore secreted. This plasmid was a generous gift from D. Margulies, of the NIH [24]. Plasmid (phβ2m) was constructed to express the human

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β2-microglobulin. It is based on the cDNA of human β2m (hβ2m) isolated from PC3 cells and amplified by PCR using the following primers: 5'-sense primer: 5'-AGATTCCCAAGCTTATGTCTCGCTCCGTGG-3' (SEQ ID NO:40) contained a restriction site for Hind III before the signal peptide and a 3' antisense primer 5'-AGCTAGTCTAGATTATCACATGTCTC GATCCCACTTAAC-3' (SEQ ID NO:41) contained the restriction site for *Xba*I on the 3' end of β2m. The purified PCR product was cut with *Hind*III and *Xba*I and ligated into the eukaryotic expression vector pCDNA-3.1 (Invitrogen). Plasmid sHLA-A2 and sHLA-B7 contains the cDNA of the first 4 exons of this alleles ligated into the plasmid pcDNA3.1 [34].

Antibodies and hybridomas:

The hybridomas W6/32 and BB7.2, an anti-MHC class-I and anti-HLA-A2 respectively, and HB-149 an anti β2m were obtained from the ATCC. The antibodies were affinity purified using protein A-Sepharose CL-4B (Sigma) from mouse ascites fluid.

Transfection of cancer cells and selection of clones secreting sMHC:

Cell lines were co-transfected with plasmid HLA-A2/Q10^b and with phβ2m, which conferred resistance to the antibiotic G418 or transfected only with the plasmids sHLA-A2 and sHLA-B7 that contained the antibiotic resistance. Cells were electroporated by use of a Gene Pulser (Bio-Rad) set at 280-300 mV 960 μF. Transfected cell clones were selected in G418 antibiotic and screened for those secreting sMHC to the growth medium. Secretion of

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sMHC was assayed by sandwich-ELISA with plates coated with the mAb BB7.2 (for sMHC-A2) or by HB-149 (for sMHC-B7) and sMHC was detected with the biotinilated mAb W6/32. Color was developed with ABTS (Sigma) catalyzed by streptavidin peroxidase (Sigma).

Affinity purification of soluble MHC:

Cultured cells, expressing the soluble MHC were grown to confluency in 150 mm plates. The culture medium was collected and residual cells were removed by centrifugation. Soluble MHC molecules were purified from the cleared culture medium by affinity chromatography on W6/32 antibody columns at 4 °C. The antibodies were coupled to NHS-activated agarose (Pharmacia) or to protein A Sepharose (Sigma) with n-methylpipelimidate (Sigma). The columns were washed with 0.5 M NaCl, 20 mM, Tris pH 8. The MHC molecules were eluted from the column with 0.1 M acetic acid at pH 3. Peptides were separated from the MHC complexes by boiling for five minutes in 10 % acetic acid followed by ultra-filtration through a 3 kDa Microcon (Amincon) [2].

Synthetic peptides:

Peptides were synthesized on AbiMed AMS 422 multiple peptide synthesizer (Abimed, Langenfeld, Germany) by Fmoc chemistry, precipitated with ether and used with or without further purification (HPLC).

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Peptides separation and analysis:

The MHC bound peptides were resolved by reverse-phase HPLC on a 0.1 ID fused silica capillaries with length of about 30 cm (J&W) slurry packed with POROS 10 R2 (PerSepetive Biosystems). The capillaries were fitted with electrospray needle made from 36-gauge stainless tubing (Small Parts Inc. Miami Lakes, FL). A Rheodyne 9125 HPLC injector fitted with a 20 μl loop was used for loading the column. The peptides were resolved by a relatively long (90 minutes) linear gradient of 5 to 50 % acetonitrile with 0.1 % acetic acid, at a flow rate of about 1 μl/minute. The flow was electrosprayed directly from the HPLC column into an ion trap mass spectrometer (LCQ, Finnigan). The mass spectrometry analysis was done in the positive ion mode, using repetitively a full MS scan usually between 450 to 1500 atomic mass units (amu) followed by collision-induced decomposition (CID) of the dominant ion selected from the previous MS scan. In some cases the full MS was performed with a narrower mass range to reduce the number of detected peptides. The peptides were identified by comparing their MS and CID data to the calculated MS and CID of the proteins in the Genpept databank (www.ncbi.nlm.nih.gov/genpept) using the Sequest software [25] (obtained from Finnigan, San Jose, CA). The number of times each peptide was fragmented by CID was usually limited to two by dynamic exclusion, a feature of the Xcalibur control software the LCQ mass spectrometer (Finnigan).

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Stabilization of cell surface HLA-2.1 by peptide binding:

RMA-S-HHD cells were washed three times with PBS followed by incubation overnight in FCS-free IMDM medium at 26°C. Synthetic peptides were added to 10^6 cells at a concentration of 100 μM. The cells were incubated for two hours at 26°C followed by two hours at 37°C. The stabilization of the HHD MHC by the peptides binding was measured by FACS analysis on Becton Dickinson FACStar flow cytometer after decorating the cells with W6/32 mAb at 4°C for one hour and then 30 min incubation with anti-mouse FITC at 4°C (Sigma).

Cytotoxic T lymphocytes assays:

Transgenic mice expressing a single chain HLA-A2.1/Db-β2m which are double knockout for H-2Db and for β2m (HHD mice) [23] were immunized four times intra-peritoneally at 7-day interval with 2 x 10^6 irradiated (5,000 rad) RMA-S-HHD-B7.1 cells loaded for two hours at 26 °C followed by two hours at 37 °C with 100 μM of the synthetic peptides. Ten days after the last immunization the spleens were removed from the vaccinated mice. Splenocytes were re-stimulated with 100 μM of synthetic peptides for five days. Viable lymphocytes were separated by lympholyte-M (Cadarlane, Hornby, Canada) and resuspended in RPMI-HEPES. Cytotoxic activity was measured as in [26] by admixing the lymphocytes at different ratios with 5 x 10^3 EL4-HHD cells grown in medium containing ^{35}S methionine and then loaded with the synthetic peptides.

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EXPERIMENTAL RESULTS

In order to identify large number of MHC bound peptide antigens presented in the context of a particular MHC haplotype, different human cell lines were transfected with expression vectors for soluble, secreted MHC molecules. Indeed, different soluble MHC could be transfected into various cell lines resulting in enabling the recovery of large amounts of the soluble MHC molecules from the cell's growth medium. The sMHC molecules were recovered with their authentic patterns of peptides still bound and free of contamination by cellular debris and detergents. Prostate (PC3), ovarian (UCI-107) and breast (MDA-231 and MCF-7) cell lines were transfected with the DNA coding HLA-A2.1/Q10^{b}, or sMHC-A2 and sMHC-B7. Soluble MHC molecules were recovered from the culture medium without disrupting the cells and the sMHC molecules were purified by a single step of immunoaffinity chromatography. About 200 μg of the sMHC molecules were recovered from about 10^9 cells (Figures 1A-C). The MHC large subunit, the β2m and small amounts of antibodies that were released from the immunoaffinity column by the acid treatment were the only proteins detected in the column eluant. The peptides were separated from the proteins subunits of the MHC by ultra-filtration. The recovered heavy subunit of the soluble MHC molecules was confirmed to be that of HLA-A2.1 by peptide mapping and by micro sequencing.

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Sequencing of a large number of individual peptides was approached by electrospray tandem mass spectrometry. The peptides were partially resolved on homemade nano-capillary reversed phase columns interfaced directly to an electrospray mass spectrometer. The peptide mixtures were resolved by relatively long reversed-phase HPLC gradients on long capillary columns, enabling performing mass measurements and fragmentation of a large number of peptides. The mass spectra were recorded between 450 to 1500 mass units, which is the expected mass (m/z) range of the singly and the doubly charged MHC bound peptides. The mass spectrometry data included the total-ion-current chromatogram (TIC, Figure 2A) and the mass spectrum of the peptides at each time point (Figure 2B). The mass spectrometer was programmed to repeatedly select the most abundant peptide observed in each spectrum and to fragment it by CID (Figure 2C). Peptides were identified by comparing their masses and the masses of their fragments to those calculated for peptides derived from all the human proteins in the databank. The computer programs were instructed to search for putative peptides resulting from non-specific proteolysis since the specificity of proteases responsible for generating the MHC bound peptides in cells is not well defined.

The relatively high sensitivity of the capillary ESI-MS/MS analysis and the large amounts of peptides recovered from the cells by use of the soluble MHC, enabled to perform multiple capillary HPLC separations with each peptide preparation. Peptides recovered from soluble MHC produced by about

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$5x10^7$ cells were used for each capillary chromatography. Multiple chromatography runs enabled to detect those peptides that were observed reproducibly and to combine their CID data to improve the signal-to-noise ratio of the CID spectra. The combined and improved data sets were used for databank searches and peptide identifications. Using relatively long capillary columns (of above 30 cm) and long reversed phase gradients facilitated achieving high resolving power. Most peptides elute normally during 15 to 30 seconds, which was a sufficient time for the mass spectrometer to analyze up to three different co-eluting peptides. The mass spectrometer was programmed not to fragment any peptide more than twice in order to increase the total number of peptides analyzed during each chromatography.

A total of about three thousands different peptides were sufficiently resolved and fragmented during the different chromatography runs of the mixtures eluted from the sHLA-A2 and sHLA-B7 recovered from the different cell lines. The large majority of the observed peptides were common to all the different cancer lines and only a small fraction was detected in only one of the cancer types. From this large number of detected peptides, about 200 were identified at high certainty to be derived from known proteins and the rest were not identified. Table 8 is typical list of such peptides recovered from the soluble MHCs and identified by the computer analysis. Among these peptides, fourteen were already known as MHC bound peptides. Those desired peptides that originate from putative tumor antigens were chemically synthesized to

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further evaluate the accuracy of their amino acid sequences and to enable to study them as MHC bound peptides and their significance as cancer antigens. Their amino acid sequence accuracy was ascertained by running a chromatography of the synthetic peptides using the exact conditions immediately after the natural peptides mixtures and comparing the chromatography retention times, the exact masses and the CID spectra of the synthetic and natural peptides (Figures 3A-B). When synthetic peptides behaved identically to the natural peptides in these three criteria served as a clear indication that the identification was indeed correct. Twenty-seven of the most interesting peptides were chemically synthesized and confirmed to be correct by this assay, example of which is displayed in Figures 3A-B.

Table 8
List of peptides recovered from sMHC of different cancer cells and identified at high certainty by mass spectrometry

Peptides from soluble HLA-A2

	Mass (m/z)	Sequence (SEQ ID NO:)	Protein	Position[1]	Score[2]	Score[3]	Synthetic[4]	Ref[5]
1	898.4	LLDVPTAAV(1)	γ IFN inducible protein (IP-30)	17-25	159.9	28		[41]
2	1011.5	LLLDVPTAAV(2)	γ IFN inducible protein (IP-30)	16-25	1793.7	31		[41]
3	1210.4	LLLDVPTAAVQA(3)	γ IFN inducible protein (IP-30)	16-27	128.1	21		[41]
4	800.5	GLLGTLVQ(4)	Beta catenin	400-407	0.2	17	+	
5	913.4	GLLGTLVQL(5)	Beta catenin	400-408	181.7	31	+	
6	922.3	ALFGALFLA(6)	Phospholipid transfer protein	2-10	245.2	23	+	
7	945.4	SLLGGDVVSV(7)	TSC-22-like protein	22-32	591.9	34	+	
8	947.4	NLTISDVSV(8)	MUC1	130-138	69.6	23	+	[26]
9	958.3	SLWGQPAEA(9)	Human collagen type IV	18-25	41.2	23	+	
10	981.7	SLIGHLQTL(10)	protein tyrosine posphatase	336-344	49.1	32	+	
11	989.5	SLSEKTVLL(11)	CD59	106-114	87.6	29	+	
12	989.4	SLFPGKLEV(12)	Flightless I homolog	1010-18	257.3	30	+	
13	1028.5	GLIEKNIEL(13)	DNA methyl transferase (MTDM)	425-433	87.6	28	+	
14	1031.4	GLYPGLIWL(14)	Interferon regulatory factor-6	21-29	864.8	30	+	
15	1038.5	YLLPAIVHI(15)	RNA helicase	146-154	408.4	30		[2]
16	1068.4	ALSDHHIYL(16)	Fructose bisphosphate aldolase	216-224	481.7	23	+	[21]
17	1071.5	ILDQKINEV(17)	ornithine decarboxylase	23-31	108.8	30		[96]
18	1071.6	ILDKKVEKV(18)	Human HSP 90 beta, HSP 84	570-578	53.3	29		[74]
19	1080.4	SLLPPTALVGL(19)	H. Transporter SEC23A	156-164	181.8	33		
20	1091.4	GVYDGEEHSV(20)	MAGE-B2	231-240	79.9	20	+	

21	1094.5	SLLPPDALVGL(21)	H, Transporter SEC23B	150-160	181.8	33		
22	1121.3	TLWVDPYEV(22)	B- cell translocation gene (BTG1)	103-111	577.3	24	+	[2]
23	1145.4	FLFDGSPTYV(23)	Fatty acid synthase (FAS)	292-301	26694	23	+	
24	1258.5	FLFDGSPTYVL(24)	Fatty acid synthase (FAS)	292-302	611.2	27	+	
25	1360.4	ALWDIETGQQTV(25)	guanine nucleotide-binding	167-178	2366.8	28	+	

Peptides from soluble HLA-B7

	Mass (m/z)	Sequence (SEQ ID NO:)	Protein	Position[1]	Score[2]	Score[3]	Synthetic[4]	Ref[5]
1	854.3	VPSEPGGVL(26)	70 kDa SHP-1L	422-30	120	27	+	
2	883.4	SPTQPIQL(27)	cell membrane glycoprotein 110000 Mr	257-61	80	20		
3	895.4	SPALPGLKL(28)	transmembrane activator and CAML interactor	147-55	120	27	+	
4	899.5	APRTVALTA(29)	HLA-SB beta	9-17	60	24		[75]
5	927.3	SPKLPVSSL(30)	DNA binding protein homolog	372-80	120	25	+	
6	989.3	KPSLPFTSL(31)	translation initiation codon	79-87	120	28	+	
7	999.5	LVMAPRTVL(32)	MHC class-I	2-10	135	18		[75]
8	1050.4	KPAFPAEKL(33)	annexin A1	274-82	80	22		
9	1075.4	SPYQNIKIL(34)	spermidine aminopropyltransferase	128-36	80	20		
10	1104.5	AASKERSGVSL(35)	Histone H1	50-60	36	18		[75]
11	1114.3	APPEPLASGIL(36)	precursor	2-12	240	22	+	
12	1194.5	APSGSLAVPLAVL(37)	hypothetical protein	9-21	360	31		

Table 8: An example of MHC bound peptides that were identified by the Sequest software [25] (obtained from Finnigan, San Jose, CA) after mass spectrometer analysis. [1]Position of the first and the last amino acid of the peptide. [2]Calculated score, estimating half the time for dissociation of the peptide–MHC complex [42]. [3] Calculate score. [4] sequence approved by analyzing in comparison a synthetic peptide. [5] Peptide is known.

Among the many peptides derived from different housekeeping proteins and enzymes, some peptides were determined to be derived from known tumor associated antigens. These include mucin (MUC-1) and MAGE-B2 while others were derived from proteins whose level is known to be significantly elevated in cancer cells such as beta-catenin, DNA methyl transferase and fatty acid synthase (Table 8).

A comparison in the patterns of peptides presented by the same MHC in cell lines of different tissue origin enabled the identification of those peptide uniquely presented in only cells of a particular tissue origin. Only a few of the

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peptides were determined to be unique to specific cell lines while most of the peptides that were observed in all the different cell lines were derived from normal cellular proteins. Also, significantly different patterns of peptides were recovered from sHLA-A2 and from sHLA-B7. Examples for unique peptides which are displayed in Table 9 include peptide p922 (phospholipid transfer protein) recovered only from PC3 cells and peptide p947 (SEQ ID NO:8) (MUC1) recovered only from MCF-7 grown without estrogen. Peptide p945 (SEQ ID NO:7, derived from TSC-22-like protein) is a novel peptide that was detected at high level in the two-breast cancer cells (MCF-7 and MDA-231), but was not observed in the ovarian (UCI-107) and the prostate (PC3) cancer cells. Peptide p981 (SEQ ID NO:10) originated from protein tyrosine phosphatase, and was detected only in the breast cancer cell MDA-231. One of the most interesting novel peptides identified was p1091 (SEQ ID NO:20) derived from the tumor antigen MAGE-B2. The peptide was detected only in the ovarian cancer cells (UCI-107) and not in the other cell lines. The synthetic and natural peptides elution pattern and CID spectra of both were identical (Figures 4A and 4B). The binding affinity of this peptide to the MHC molecules was determined to be normal as assayed by reconstitution and stabilization of empty MHC on the surface of RMA-S-HHD cells (Figure 4C). This peptide is derived from the same region in the MAGE proteins, as do other previously identified MHC bound peptides derived from MAGE-A4 and from MAGE-A10 [27, 28] (Figure 4D).

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Table 9
Comparison of MHC peptide patterns between cell lines of different cancer origin

(A)

	Mass (m/z)	Sequence (SEQ ID NO.)	MCF-7	[1] MCF-7 without estrogen	MDA-231	PC-3	UCI-107	UCI-101
1	898.4	LLDVPTAAV(1)	+	+	+	+	+	+
2	1011.5	LLLDVPTAAV(2)	+	+	+	-	+	+
3	1210 5	LLLDVPTAAVQA(3)	+	+	+	+	+	+
4	800 5	GLLGTLVQ(4)	-	-	-	-	+	-
5	913.4	GLLGTLVQL(5)	+	+	+	+	+	+
6	922 3	ALFGALFLA(6)	-	-	-	+	-	-
7	945.4	SLLGGDVVSV(7)	+	+	+	-	-	-
8	947.4	NLTISDVSV(8)	-	+	-	-	-	-
9	958.3	SLWGQPAEA(9)	+	+	-	+	+	+
10	981.7	SLIGHLQTL(10)	-	-	+	-	-	-
11	989.5	SLSEKTVLL(11)	+	+	+	-	+	+
12	989.4	SLFPGKLEV(12)	+	+	+	+	+	+
13	1028.5	GLIEKNIEL(13)	+	+	+	+	+	+
14	1031 4	GLYPGLIWL(14)	+	+	+	+	-	+
15	1038.5	YLLPAIVHI(15)	+	+	+	+	+	+
16	1068 4	ALSDHHIYL(16)	+	+	+	+	+	+
17	1071.5	ILDQKINEV(17)	-	+	+	+	+	-
18	1071.6	ILDKKVEKV(18)	-	+	+	+	+	+
19	1080 4	SLLPPTALVGL(19)	-	-	+	-	+	+
20	1091.4	GVYDGREHSV(20)	-	-	-	-	+	-
21	1094.4	SLLPPDALVGL(21)	+	+	+	-	+	+
22	1121.3	TLWVDPYEV(22)	+	+	+	+	+	+
23	1145.4	FLFDGSPTYV(23)	+	-	+	-	+	-
24	1258.5	FLFDGSPTYVL(24)	+	+	+	-	+	+
25	1360.4	ALWDIETGQQTV(25)	-	-	+	-	+	-

(B)

	Mass (m/z)	Sequence (SEQ ID NO:)	C1R	MDA-231	UCI-107
1	854.3	VPSEPGGVL(26)	+	-	-
2	883.4	SPTQPIQL(27)		+	-
3	895 4	SPALPGLKL(28)	+	-	-
4	899.4	APRTVALTA(29)	+	-	-
5	999 5	SPKLPVSSL(30)	+	+	+
6	927.3	KPSLPFTSL(31)	+	-	+
7	989 3	LVMAPRTVL(32)	+	-	-
8	1050.4	KPAFFAEKL(33)	-	-	+
9	1075.4	SPYQNIKIL(34)	-	+	-
10	1104 5	AASKERSGVSL(35)	+	-	+
11	1114.3	APFEPLASGIL(36)	+	+	+
12	1194.5	APSGSLAVPLAVL(37)	-	+	-

Table 9: (A) The appearance of peptides from soluble HLA-A2 in breast cancer cells MCF-7 and MDA-231, [1] MCF-7 that grown without estrogen, prostate cancer cell PC-3 and the ovarian cancer cells UCI-107 and UCI-101. (B) The appearance of peptides from soluble HLA-B7 in B cell leukemia

cancer cells C1R, breast cancer cells MDA-231 and ovarian cancer cells UCI-107.

Another approach to ascertain that the identified peptides were indeed MHC bound peptide antigens, their capacity to bind tightly and stabilize cell surface HLA-A2.1 was tested by reconstitution into empty MHC on the surface of RMA-S-HHD cells. Binding was assayed by FACS analysis after decorating the cells with the fluorescent anti-intact MHC mAb W6/32 (Figure 5). Nine of the synthetic peptides were determined to stabilize cell surface MHC significantly more than without the added peptides and to a similar extent as peptide (G9-209-2M) IMDQVPFSV (SEQ ID NO:42), derived from the melanoma protein gp-100 [29].

To further evaluate the affinity of the peptides to the HLA-A2 and to obtain some insight into their immunogenic potential, selected peptides were tested for their ability to induce an immune response in HLA-A2 transgenic mice. It was assumed that only peptides that could be effectively presented and remain tightly bound to the cells would be capable of inducing an immune response in these mice. The same synthetic peptides that were used for the FACS analysis were used for immunization of the HHD transgenic mice, which express the human HLA-A2.1/Db- β2m single chain. To immunize the mice, the HHD culture cells were loaded with the different peptides and then injected to the HHD mice. The immune response in the mice was followed by the appearance of CTLs specific for these peptides. The lysis patterns of the target HHD cells by T-cells taken from the immunized mice are shown in

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Figure 6. Some of the peptides were indeed capable of inducing an immune response, which both authenticate them as MHC bound peptides and gives an indication about their immunogenic potential. The CTL results demonstrate significant lysis of EL4-HHD cells loaded with the peptides p1028 (SEQ ID NO:13) from DNA methyl transferase, p1258 (SEQ ID NO:24) from fatty acid synthase, p1121 (SEQ ID NO:22) from B cell translocation gene (BTG) and p1068 (SEQ ID NO:16) from aldolase as compared to the negative control peptide ALLCAPSLL (SEQ ID NO:43).

SUMMARY OF PEPTIDE INFORMATION FOR SOLUBLE HLA-A2

The following provides a summary of peptide information so far collected for eptides bound to soluble HLA-A2 using the method of the present invention.

The following notations are used herein:

G:	group number until May 7, 2001
Mg:	mass of the natural peptide
Mp:	mass of the identified peptide
Tg:	observed retention time of the natural peptide
Tp:	calculated retention time of the identified peptide
S:	calculated internal score
A2:	adherence to HLA-A2 consensus motif
B7:	adherence to HLA-B7 consensus motif
P:	identified peptide sequence
PR:	protein from which sequence is derived
POS:	location of peptide in protein
genpept:	link to protein information in GenBank
ref:	previously known peptide

Cell lines:

#D:	PC3+A2/Q10
#E:	MCF7+A2/Q10
#F:	MDA-231+A2/Q10
#EST:	MCF7(with estrogen)+A2/Q10
#FR:	MCF7(without estrogen)+A2/Q10
#G:	UCI-107+A2/Q10
#H:	C1R+sB7
#I:	UCI-107+sB7

#J: MDA-231+sB7
#K: UCI-101+sA2
#L: 2780 (ovarian cancer cell line)+sA2
#S: synthetic peptides

G=1990: Mg= 800.4: #S+(2,1) #G+(10,7)S=83(87,74) Mg= 800.5 Tg=38+-0 Tp= 53 Mp=800.5 A2= 0.02/18 P=GLLGTLVQ
genpept PR=>gi|860988|emb|CAA61107.1| (X87838) beta-catenin [Homo sapiens] POS=399 (SEQ ID NO:4)

G=1234: Mg= 810.3: Tg=31+-1 #D+(2,2) #E+(7,4) #F+(4,3) #EST+(7,2) #FR+(4,2) #G+(33,11) #K+(3,2) #L+(2,1)
S=84(87,79) Mp= 810.2(-0.1) Tp= 34 A2= 11/29 P=ALAPGLPTA
genpept PR=>gi|5771535|gb|AAD51419.1|AF173937_1 (AF173937) secreted protein of unknown function [Homo sapiens] POS=21 (SEQ ID NO:44)

G=1251: Mg= 811.4: Tg=35+-0 #G+(5,3)
S=96(96,99) Mp= 811.5(0.1) Tp= 36 A2=465/26 P=KLLEPVL
genpept PR=>gi|338447|gb|AAA60583.1| (M60854) RPS16 [Homo sapiens] POS=50 (SEQ ID NO:45)

G=1378: Mg= 841.3: Tg=41+-0 #G+(9,5)
S=77(83,66) Mp= 841.4(0.1) Tp= 40 A2=0.0/16 P=SLLPAIVE
genpept PR=>gi|189428|gb|AAA36399.1| (J02902) phosphatase 2A regulatory subunit [Homo sapiens] POS=403 (SEQ ID NO:46)

G=1419: Mg= 848.3: Tg=34+-1 #E+(2,1) #F+(2,1) #G+(5,5) #K+(1,1)
S=84(83,89) Mp= 848.4(0.1) Tp= 34 A2= 52/26 P=SVLGSLSSV
genpept PR=>gi|5833114|gb|AAD53401.1|AF107840_1 (AF107840) nuclear pore-associated protein [Homo sapiens] POS=280 (SEQ ID NO:47)

G=1420: Mg= 848.4: Tg=37+-1 #D+(2,2) #E+(11,6) #F+(8,5) #EST+(3,2) #FR+(4,2) #G+(25,11)
S=95(94,99) Mp= 848.4(0.0) Tp= 39 A2=118/28 P=LLGPPPVGV
genpept PR=>gi|10436199|dbj|BAB14750.1| (AK023978) unnamed protein product [Homo sapiens] POS=159 (SEQ ID NO:48)

G=1439: Mg= 852.3: Tg=22+-2 #FR+(2,1) #I+(32,6)
S=83(81,89) Mp= 852.0(-0.3) Tp= 25 A2-0.0/1 P-FGPPPPPPP
genpept PR=>gi|5689367|dbj|BAA82967.1| (AB021227) membrane-type-5 matrix metalloproteinase [Homo sapiens] POS=11 (SEQ ID NO:49)

G=1492: Mg= 860.3: Tg=37+-1 #G+(9,5)
S=81(87,69) Mp= 860.3(0.0) Tp= 31 A2=116/28 P=SMSGPLIGV
genpept PR=>gi|1469189|dbj|BAA09482.1| (D50923) The KIAA0133 gene product is novel. [Homo sapiens] POS=629 (SEQ ID NO:50)

G=1510: Mg= 862.5: Tg=29+-2 #F+(4,4) #G+(4,2)
S=78(87,59) Mp= 862.2(-0.3) Tp= 27 A2=116/32 P=SMAPGLTSV
genpept PR=>gi|12484559|gb|AAF20366.2|AF150754_1 (AF150754) 3'phosphoadenosine 5'-phosphosulfate synthase 2b isoform [Homo sapiens] POS=542 (SEQ ID NO:51)

G=1540: Mg= 868.4: Tg=43+-0 #E+(2,1) #F+(7,4) #FR+(1,1) #G+(4,4) #K+(3,3)

S=83(89,69) Mp= 868.4(0.0) Tp= 46 A2= 19/30 P=LLIPGLATA genpept PR=>gi|2274974|emb|CAA57489.1| (X81900) NADH oxidoreductase subunit MWFE [Homo sapiens] POS=16 (SEQ ID NO:52)

G=1563: Mg= 871.3: Tg=36+-1 #D+(1,1) #E+(8,6) #F+(4,3) #EST+(3,2) #FR+(5,2) #G+(15,9)
S=86(89,79) Mp= 871.4(0.1) Tp= 33 A2=592/33 P=GLLGNVAEV genpept PR=>gi|12655181|gb|AAH01447.1|AAH01447 (BC001447) Similar to ZYG homolog [Homo sapiens] POS=10 (SEQ ID NO:53)

G=1575: Mg= 872.4: Tg=32+-3 #D+(2,1) #E+(3,3) #F+(24,7) #EST+(3,2) #FR+(4,2) #G+(12,7)
S=79(76,88) Mp= 872.5(0.1) Tp= 33 A2= 11/26 P=SLIKLVEA genpept PR=>gi|7020538|dbj|BAA91170.1| (AK000444) unnamed protein product [Homo sapiens] POS=277 (SEQ ID NO:54)

G=1606: Mg= 876.4: Tg=28+-2 #E+(2,1) #F+(2,1) #EST+(1,1) #FR+(4,2)
S=84(82,89) Mp= 876.3(-0.1) Tp= 32 A2=201/31 P=GLAESVSTL genpept PR=>gi|12652733|gb|AAH00116.1|AAH00116 (BC000116) Similar to KIAA0174 gene product [Homo sapiens] POS=95 (SEQ ID NO:55)

G=1621: Mg= 878.3: Tg=40+-1 #E+(6,4) #F+(4,2) #EST+(1,1) #FR+(3,2) #G+(11,8) #K+(1,1)
S=88(92,79) Mp= 878.4(0.1) Tp= 40 A2= 55/30 P=AIIGGTFTV genpept PR=>gi|6330243|dbj|BAA86495.1| (AB033007) KIAA1181 protein [Homo sapiens] POS=304 (SEQ ID NO:56)

G=1637: Mg= 880.3: Tg=37+-0 #H+(5,3)
S=88(89,88) Mp= 880.4(0.1) Tp= 40 A2= 2/23 P=IITGPAPVL genpept PR=>gi|7542357|gb|AAF63417.1|AF142422_1 (AF142422) QUAKING isoform 3 [Homo sapiens] POS=250 (SEQ ID NO:57)

G=1655: Mg= 882.3: Tg=43+-1 #F+(1,1) #G+(6,4) #K+(1,1)
S=87(87,88) Mp= 882.3(0.0) Tp= 43 A2=0.0/18 P=SFDGWATV genpept PR=>gi|7263944|emb|CAB81773.1| (AJ276359) mucin 4 [Homo sapiens] POS=1560 (SEQ ID NO:58)

G=1732: Mg= 894.4: Tg=42+-0 #G+(6,3)
S=79(82,72) Mp= 894.4(0.0) Tp= 41 A2= 2/20 P=LPPDALVGL genpept PR=>gi|1296666|emb|CAA65775.1| (X97065) Sec23 protein [Homo sapiens] POS=158 (SEQ ID NO:59)

G=1737: Mg= 895.3: Tg=14+-1 #G+(4,2)
S=76(79,72) Mp= 895.4(0.1) Tp= 22 A2= 47/25 P=ILDAGGHNV genpept PR=>gi|1808578|dbj|BAA07918.1| (D44466) proteasome subunit p112 [Homo sapiens] POS=736 (SEQ ID NO:60)

G=1744: Mg= 896.3: Tg=28+-3 #D+(6,3) #E+(14,5) #F+(20,6) #EST+(5,2) #FR+(6,3) #G+(47,11) #K+(8,4)
S=92(98,81) Mp= 896.4(0.1) Tp= 30 A2=512/30 P=GLYSGVTTV genpept PR=>gi|36065|emb|CAA42118.1| (X59543) M1 subunit of ribonucleotide reductase [Homo sapiens] POS=46 (SEQ ID NO:61)

G=1745: Mg= 896.3: Tg=55+-0 #G+(8,4)
S=79(76,86) Mp= 896.5(0.2) Tp= 60 A2=>1k/24 P=FLYPFPL genpept PR=>gi|436224|dbj|BAA05062.1| (D26067) KIAA0033 [Homo sapiens] POS=185 (SEQ ID NO:62)

G=1768: Mg= 898.4: Tg=36+-1 #D+(11,3) #E+(50,10) #F+(13,7) #EST+(7,2) #FR+(13,3) #G+(63,11) #K+(15,6) #L+(9,3)
S=81(78,89) Mp= 898.4(0.0) Tp= 36 A2= 47/28 P=LLDVPTAAV
genpept PR=>gi|6165618|gb|AAF04618.1|AF097362_1 (AF097362) gamma-interferon inducible lysosomal thiol reductase [Homo sapiens] POS=27 (SEQ ID NO:1) ref

G=1770: Mg= 898.4: Tg=38+-1 #E+(4,2) #F+(3,2) #FR+(1,1) #G+(18,11) #K+(2,1)
S=88(92,79) Mp= 898.3(-0.1) Tp= 41 A2= 79/29 P=ALLPSSPTL
genpept PR=>gi|1737205|gb|AAB38876.1| (U75276) TFIIB related factor hBRF [Homo sapiens] POS=609 (SEQ ID NO:63)

G=1786: Mg= 899.5: Tg=26+-2 #F+(2,2) #EST+(4,2) #FR+(5,2) #G+(32,8) #K+(8,5) #L+(1,1)
S=93(96,89) Mp= 899.5(0.0) Tp= 27 A2=243/25 P=KLGSVPVTV
genpept PR=>gi|12653653|gb|AAH00609.1|AAH00609 (BC000609) KIAA0738 gene product [Homo sapiens] POS=623 (SEQ ID NO:64)

G=1795: Mg= 900.4: Tg=53+-0 #D+(2,2) #E+(7,5) #F+(11,6) #EST+(2,1) #FR+(6,3) #G+(21,11) #K+(16,6)
S=81(86,72) Mp= 900.5(0.1) Tp= 55 A2=182/33 P=ALFPGVALL
genpept PR=>gi|2245365|gb|AAC51518.1| (U75685) ER-60 protein [Homo sapiens] POS=7 (SEQ ID NO:65)

G=1802: Mg= 901.3: Tg=33+-2 #E+(3,2) #G+(2,1)
S=85(92,69) Mp= 901.5(0.2) Tp= 32 A2=160/29 P=GLVGSLQEV
genpept PR=>gi|11967711|emb|CAC19484.1| (AJ278357) Tsg24 protein [Homo sapiens] POS=56 (SEQ ID NO:66)

G=1804: Mg= 901.4: Tg=31+-2 #E+(1,1) #FR+(1,1) #G+(6,3)
S=90(95,79) Mp= 901.3(-0.1) Tp= 22 A2= 2/18 P=APLSDTAQV
genpept PR=>gi|10438789|dbj|BAB15344.1| (AK026063) unnamed protein product [Homo sapiens] POS=197 (SEQ ID NO:67)

G=1804: Mg= 901.4: Tg=31+-2 #E+(1,1) #FR+(1,1) #G+(6,3)
S=89(94,79) Mp= 901.5(0.1) Tp= 36 A2=160/33 P=SLASLLAKV
genpept PR=>gi|8489831|gb|AAF75772.1|AF265555_1 (AF265555) ubiquitin-conjugating BIR-domain enzyme APOLLON [Homo sapiens] POS=1230 (SEQ ID NO:68)

G=1822: Mg= 903.3: Tg=16+-7 #D+(7,3) #E+(14,4) #F+(14,4) #EST+(18,2) #FR+(13,3) #G+(116,10) #K+(20,6)
S=92(98,79) Mp= 903.4(0.1) Tp= 23 A2=160/29 P=GLATDVQTV
genpept PR=>gi|565647|dbj|BAA05645.1| (D26598) proteasome subunit HsC10-II [Homo sapiens] POS=55 (SEQ ID NO:69)

G=1860: Mg= 907.5: Tg=39+-1 #D+(12,4) #E+(1,1) #F+(9,5) #EST+(3,1) #FR+(4,2) #G+(10,6) #K+(4,3)
S=88(91,81) Mp= 907.5(0.0) Tp= 37 A2= 79/31 P=SLFGGSVKL
genpept PR=>gi|13375569|gb|AAK20398.1|AF349951_1 (AF349951) HP95 [Homo sapiens] POS=103 (SEQ ID NO:70)

G=1861: Mg= 907.6: Tg=38+-1 #EST+(2,1) #FR+(1,1)

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S=87(87,89) Mp= 907.6(0.0) Tp= 32 A2= 21/19 P=KVGPVPVLV
genpept PR=>gi|12804623|gb|AAH01734.1|AAH01734 (BC001734) protein translocation complex beta [Homo sapiens] POS=67 (SEQ ID NO:71)

G=1899: Mg= 910.3: Tg=46+-1 #E+(8,5) #F+(21,7) #EST+(4,2) #FR+(7,3) #G+(24,11) #K+(13,6) #L+(3,2)
S=72(65,89) Mp= 910.4(0.1) Tp= 40 A2=182/32 P=GLLPDVPSL
genpept PR=>gi|13623421|gb|AAH06309.1|AAH06309 (BC006309) Similar to RIKEN cDNA 5730589L02 gene [Homo sapiens] POS=141 (SEQ ID NO:72)

G=1901: Mg= 910.4: Tg=39+-0 #D+(2,1) #E+(5,2) #F+(9,5) #EST+(1,1) #FR+(1,1)
S=80(90,59) Mp= 910.4(0.0) Tp= 41 A2=160/30 P=ALPPVLTTV
genpept PR=>gi|3882183|dbj|BAA34451.1| (AB018274) KIAA0731 protein [Homo sapiens] POS=131 (SEQ ID NO:73)

G=1904: Mg= 910.4: Tg=38+-1 #E+(2,1) #F+(3,2) #EST+(1,1) #FR+(3,2) #G+(3,3) #K+(2,1)
S=90(95,79) Mp= 910.5(0.1) Tp= 32 A2= 52/24 P=GVLPNIQAV
genpept PR=>gi|7264004|emb|CAB81656.1| (AL049822) dJ160A22.4 (histone H2A) [Homo sapiens] POS=107 (SEQ ID NO:74)

G=1922: Mg= 912.5: Tg=42+-1 #E+(5,4) #F+(2,1) #FR+(1,1) #G+(3,2) #K+(1,1)
S=78(83,69) Mp= 912.5(0.0) Tp= 43 A2= 49/31 P=ALTPVVVTL
genpept PR=>gi|13177739|gb|AAH03644.1|AAH03644 (BC003644) cyclin-dependent kinase 4 [Homo sapiens] POS=170 (SEQ ID NO:75)

G=1931: Mg= 913.4: Tg=34+-1 #E+(8,4) #F+(2,2) #EST+(3,2) #FR+(1,1)
S=84(96,59) Mp= 913.3(-0.1) Tp= 29 A2= 70/27 P=ALNPADITV
genpept PR=>gi|6634421|emb|CAB64373.1| (AJ238375) putative protein TH1 [Homo sapiens] POS=103 (SEQ ID NO:76)

G=1933: Mg= 913.4: Tg=49+-0 #S+(12,2) #D+(17,5) #E+(16,8) #F+(18,7) #EST+(2,1) #FR+(4,2) #G+(22,11) #H+(1,1) #K+(10,6)
S=93(95,89) Mp= 913.6(0.2) Tp= 46 A2=182/31 P=GLLGTLVQL
genpept PR=>gi|38520|emb|CAA79497.1| (Z19054) beta catenin [Homo sapiens] POS=400 (SEQ ID NO:5)

G=1939: Mg= 914.4: Tg=40+-0 #G+(4,3)
S=82(79,89) Mp= 914.4(0.0) Tp= 42 A2=0.0/16 P=DAEGLALLL
genpept PR=>gi|1060907|dbj|BAA11242.1| (D78177) quinolinate phosphoribosyl transferase [Homo sapiens] POS=2 (SEQ ID NO:77)

G=1942: Mg= 914.5: Tg=16+-3 #F+(4,1) #G+(6,3)
S=90(95,79) Mp= 914.4(-0.1) Tp= 27 A2=160/29 P=SLTGHISTV
genpept PR=>gi|2832296|gb|AAD09407.1| (AF044333) pleiotropic regulator 1 [Homo sapiens] POS=241 (SEQ ID NO:78)

G=1948: Mg= 915.5: Tg=38+-0 #D+(2,1) #F+(11,7)
S=91(97,77) Mp= 915.6(0.1) Tp= 42 A2=0.5/15 P=VHVLTFTV
genpept PR=>gi|3242214|emb|CAA07243.1| (AJ006778) DRIM protein [Homo sapiens] POS=1898 (SEQ ID NO:79)

G=1974: Mg= 918.3: Tg=36+-1 #F+(3,2) #G+(19,10)

S=84(88,77) Mp= 918.6(0.3) Tp= 34 A2= 6/25 P=SLKYVPLV
genpept PR=>gi|10436278|dbj|BAB14783.1| (AK024024) unnamed protein product [Homo sapiens] POS=248 (SEQ ID NO:70)

G=1979: Mg= 918.6: Tg=53+-0 #E+(5,3) #F+(6,3) #EST+(1,1) #FR+(4,2) #G+(4,2) #K+(4,3)
S=81(84,74) Mp= 918.5(-0.1) Tp= 54 A2=0.8/16 P=LPYWGVAL
genpept PR=>gi|7023639|dbj|BAA92035.1| (AK002014) unnamed protein product [Homo sapiens] POS=272 (SEQ ID NO:71)

G=1988: Mg= 920.3: Tg=32+-1 #E+(2,2) #F+(5,2) #FR+(1,1) #G+(35,11) #K+(1,1)
S=89(90,89) Mp= 920.3(0.0) Tp= 27 A2= 31/24 P=SIYPSPTGV
genpept PR=>gi|3661610|gb|AAC61776.1| (AF092565) splicing factor Prp8 [Homo sapiens] POS=1693 (SEQ ID NO:72)

G=2008: Mg= 922.3: Tg=58+-1 #S+(9,1) #D+(17,5) #L+(8,2)
S=79(80,77) Mp= 922.5(0.3) Tp= 59 A2=245/22 P=ALFGALFLA
genpept PR=>gi|2653432|dbj|BAA23647.1| (AB005297) BAI 1 [Homo sapiens] POS=1163 (SEQ ID NO:6)

G=2023: Mg= 924.2: Tg=15+-14 #F+(6,3) #FR+(1,1)
S=83(86,79) Mp= 924.4(0.2) Tp= 33 A2= 11/24 P=ALASHLIEA
genpept PR=>gi|7212807|gb|AAF40470.1|AF181263_1 (AF181263) EH domain containing 2 [Homo sapiens] POS=507 (SEQ ID NO:73)

G=2027: Mg= 924.5: Tg=13+-1 #G+(3,2)
S=83(85,79) Mp= 924.4(-0.1) Tp= 20 A2= 75/24 P=KLGPAPKTL
genpept PR=>gi|408198|gb|AAB27691.1| (S64671) DNA-binding protein/plasminogen activator inhibitor-1 regulator [human, HeLa S3, Peptide Partial, 176 aa] [Homo sapiens] POS=133 (SEQ ID NO:74)

G=2029: Mg= 924.5: Tg=43+-1 #F+(1,1) #G+(17,8)
S=93(91,99) Mp= 924.6(0.1) Tp= 44 A2=>1k/27 P=KLLEPVLL
genpept PR=>gi|338447|gb|AAA60583.1| (M60854) RPS16 [Homo sapiens] POS=50 (SEQ ID NO:75)

G=2050: Mg= 926.5: Tg=14+-3 #F+(8,2) #EST+(1,1) #FR+(3,2) #G+(6,3)
S=90(96,79) Mp= 926.4(-0.1) Tp= 29 A2= 78/30 P=ALSGHLETV
genpept PR=>gi|12314197|emb|CAB99342.1| (AL139008) bA255A11.8 (novel protein similar to annexin A2 (ANXA2) (lipocortin II, calpactin I heavy chain, chromobindin 8, PAP-IV)) [Homo sapiens] POS=90 (SEQ ID NO:76)

G=2068: Mg= 929.5: Tg=43+-1 #E+(2,2) #F+(20,7) #FR+(1,1) #G+(36,11) #K+(24,6) #L+(3,2)
S=92(90,99) Mp= 929.5(0.0) Tp= 31 A2=173/25 P=SLLDKIIGA
genpept PR=>gi|11034809|gb|AAG27093.1|AF312393_1 (AF312393) leucine-zipper protein FKSG13 [Homo sapiens] POS=56 (SEQ ID NO:77)

G=2071: Mg= 930.3: Tg=35+-1 #F+(4,3) #G+(13,7) #K+(3,2)
S=91(97,77) Mp= 930.4(0.1) Tp= 33 A2=257/33 P=GLLGAGGTVSV
genpept PR=>gi|11493522|gb|AAG35534.1|AF130117_68 (AF130109) PRO1512 [Homo sapiens] POS=17 (SEQ ID NO:78)

G=2072: Mg= 930.4: Tg=53+-0 #D+(8,4) #E+(21,8) #F+(10,5) #EST+(7,2) #FR+(8,3) #G+(11,7) #K+(7,4)

S=78(83,69) Mp= 930.6(0.2) Tp= 53 A2=608/32 P=GLVPFLVSV
genpept PR=>gi|13543657|gb|AAH05978.1|AAH05978 (BC005978) karyopherin alpha 2 (RAG cohort 1, importin alpha 1) [Homo sapiens] POS=377 (SEQ ID NO:79)ref

G=2095: Mg= 932.5: Tg=46+-0 #F+(13,7) #G+(1,1) #K+(2,1)
S=72(74,69) Mp= 932.5(0.0) Tp= 46 A2= 54/27 P=ILGLGYPSL
genpept PR=>gi|7339520|emb|CAB82850.1| (AJ250717) procathepsin E [Homo sapiens] POS=184 (SEQ ID NO:80)

G=2126: Mg= 936.3: Tg=39+-1 #E+(1,1) #F+(7,5) #G+(1,1)
S=81(86,72) Mp= 936.4(0.1) Tp= 40 A2=213/26 P=ALLAGSEYL
genpept PR=>gi|12653123|gb|AAH00328.1|AAH00328 (BC000328) eukaryotic translation initiation factor 3, subunit 7 (zeta, 66/67kD) [Homo sapiens] POS=439 (SEQ ID NO:81)

G=2146: Mg= 938.3: Tg=38+-0 #FR+(2,1)
S=77(72,90) Mp= 938.5(0.2) Tp= 34 A2=656/33 P=SLAELVHAV
genpept PR=>gi|4092863|gb|AAD04812.1| (AF033122) non-p53 regulated PA26-T1 nuclear protein [Homo sapiens] POS=254 (SEQ ID NO:82)

G=2160: Mg= 940.4: Tg=58+-1 #E+(6,4) #F+(8,3) #G+(1,1) #K+(3,2)
S=82(80,88) Mp= 940.6(0.2) Tp= 53 A2= 8/15 P=MQPILLLL
genpept PR=>gi|181159|gb|AAB59528.1| (J03072) serine protease B [Homo sapiens] POS=1 (SEQ ID NO:83)

G=2176: Mg= 942.1: Tg=48+-0 #G+(5,3)
S=74(74,77) Mp= 942.5(0.4) Tp= 48 A2= 2/12 P=GLFAPQFY
genpept PR=>gi|2062371|gb|AAB65850.1| (U70730) SnoN2 [Homo sapiens] POS=274 (SEQ ID NO:84)

G=2208: Mg= 944.5: Tg=59+-2 #S+(2,1) #L+(4,2)
S=86(94,69) Mp= 944.5(0.0) Tp= 48 A2=577/25 P=ALWGQGTLV
genpept PR=>gi|773628|gb|AAA88873.1| (U21267) immunoglobulin mu heavy chain [Homo sapiens] POS=103 (SEQ ID NO:85)

G=2213: Mg= 945.4: Tg=37+-1 #S+(12,1) #E+(60,10) #F+(6,5) #EST+(5,2) #FR+(11,3) #K+(1,1)
S=87(97,66) Mp= 945.5(0.1) Tp= 34 A2=592/34 P=SLLGGDVVSV
genpept PR=>gi|5231131|gb|AAD41085.1|AF153603_1 (AF153603) TSC-22 related protein [Homo sapiens] POS=27 (SEQ ID NO:7)

G=2231: Mg= 947.3: Tg=41+-1 #E+(4,2) #FR+(1,1) #G+(11,6)
S=85(88,81) Mp= 947.3(0.0) Tp= 20 A2=0.0/18 P=DTETAVVNV
genpept PR=>gi|4883681|gb|AAD31596.1|AF057352_1 (AF057352) hepatocellular carcinoma autoantigen [Homo sapiens] POS=117 (SEQ ID NO:86)

G=2233: Mg= 947.4: Tg=34+-1 #S+(9,1) #EST+(3,2) #FR+(4,2)
S=90(90,90) Mp= 947.4(0.0) Tp= 30 A2= 70/23 P=NLTISDVSV
genpept PR=>gi|541680|emb|CAA56734.1| (X80761) MUC1 [Homo sapiens] POS=133 (SEQ ID NO:8) ref

G=2241: Mg= 948.3: Tg=58+-1 #E+(6,3) #F+(4,3) #EST+(1,1) #FR+(3,2) #K+(5,3)

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S=75(74,79) Mp= 948.5(0.2) Tp= 62 A2=203/21 P=ALLPIFFGA genpept PR=>gi|13185197|emb|CAC33282.1| (AX083359) unnamed protein product [Homo sapiens] POS=43 (SEQ ID NO:87)

G=2270: Mg= 951.6: Tg=40+-1 #D+(11,4) #E+(49,10) #F+(2,1) #EST+(3,1) #FR+(7,3) #G+(11,6) #K+(4,2) #L+(4,2)
S=88(93,79) Mp= 951.5(-0.1) Tp= 33 A2=191/22 P=AMVIFKSGV genpept PR=>gi|3929529|gb|AAC82612.1| (AF034611) intrinsic factor-B12 receptor precursor; cubilin [Homo sapiens] POS=3371 (SEQ ID NO:88)

G=2299: Mg= 954.4: Tg=50+-0 #D+(1,1) #E+(31,9) #F+(15,7) #EST+(5,2) #FR+(8,3) #G+(28,11) #K+(16,6) #L+(4,2)
S=81(89,63) Mp= 954.5(0.1) Tp= 49 A2=182/34 P=SLLPAIVEL genpept PR=>gi|3603418|gb|AAC63525.1| (AF083439) protein phosphatase 2A regulatory subunit A, beta isoform [Homo sapiens] POS=415 (SEQ ID NO:89)ref

G=2329: Mg= 956.6: Tg=33+-2 #EST+(3,2) #FR+(5,3)
S=81(83,79) Mp= 956.5(-0.1) Tp= 32 A2=736/32 P=YLGPHIASV genpept PR=>gi|12052942|emb|CAB66646.1| (AL136711) hypothetical protein [Homo sapiens] POS=137 (SEQ ID NO:89)

G=2344: Mg= 958.3: Tg=33+-2 #S+(6,2) #D+(8,2) #E+(100,8) #G+(34,11) #K+(4,2)
S=96(96,99) Mp= 958.3(0.0) Tp= 38 A2= 41/23 P=SLWGQPAEA genpept PR=>gi|463430|gb|AAC27816.1| (U04520) type IV collagen alpha 5 chain [Homo sapiens] POS=18 (SEQ ID NO:9)

G=2350: Mg= 959.3: Tg=46+-1 #G+(14,7) #K+(8,5)
S=79(90,54) Mp= 959.6(0.3) Tp= 45 A2= 16/27 P=SLFPGQVVI genpept PR=>gi|12654999|gb|AAH01347.1|AAH01347 (BC001347) polymerase (DNA-directed), alpha (70kD) [Homo sapiens] POS=295 (SEQ ID NO:90)

G=2355: Mg= 959.5: Tg=38+-0 #F+(3,2)
S=82(80,89) Mp= 959.5(0.0) Tp= 38 A2=324/29 P=SLLEKSLGL genpept PR=>gi|13529002|gb|AAH05291.1|AAH05291 (BC005291) eukaryotic translation elongation factor 1 epsilon 1 [Homo sapiens] POS=8 (SEQ ID NO:91)

G=2356: Mg= 959.5: Tg=30+-1 #D+(3,1) #E+(29,9) #F+(2,2) #EST+(7,2) #FR+(9,3) #G+(49,10) #K+(6,2)
S=85(84,90) Mp= 959.5(0.0) Tp= 27 A2=485/24 P=ILTDITKGV genpept PR=>gi|181969|gb|AAA50388.1| (M19997) elongation factor 2 [Homo sapiens] POS=161 (SEQ ID NO:92)

G=2372: Mg= 960.5: Tg=35+-1 #D+(2,1) #G+(5,5)
S=78(73,90) Mp= 960.5(0.0) Tp= 34 A2= 79/24 P=GLFQGKTPL genpept PR=>gi|4589929|dbj|BAA76931.1| (AB024704) fls353 [Homo sapiens] POS=53 (SEQ ID NO:93)

G=2382: Mg= 962.3: Tg=47+-0 #G+(5,4)
S=80(82,77) Mp= 962.5(0.2) Tp= 11 A2=0.0/6 P=ESQLKKMV genpept PR=>gi|12803337|gb|AAH02487.1|AAH02487 (BC002487) tumor susceptibility gene 101 [Homo sapiens] POS=5 (SEQ ID NO:94)

G=2434: Mg= 967.3: Tg=54+-0 #G+(10,7)

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S=83(85,79) Mp= 967.5(0.2) Tp= 61 A2=139/19 P=FLYPFPLA genpept PR=>gi|436224|dbj|BAA05062.1| (D26067) KIAA0033 [Homo sapiens] POS=185 (SEQ ID NO:95)

G=2446: Mg= 968.4: Tg=20+-2 #F+(4,2) #G+(19,6)
S=86(89,79) Mp= 968.4(0.0) Tp= 29 A2= 78/29 P=ALTGHLEEV genpept PR=>gi|34388|emb|CAA29338.1| (X05908) lipocortin (AA 1-346) [Homo sapiens] POS=99 (SEQ ID NO:96)

G=2447: Mg= 968.4: Tg=42+-1 #D+(1,1) #E+(1,1) #F+(11,7) #FR+(1,1) #G+(27,11) #K+(6,3) #L+(3,2)
S=87(87,90) Mp= 968.4(0.0) Tp= 36 A2=>1k/33 P=SLLDPVPEV genpept PR=>gi|1504020|dbj|BAA13209.1| (D86973) similar to Yeast translation activator GCN1 (P1:A48126) [Homo sapiens] POS=1406 (SEQ ID NO:97)

G=2464: Mg= 969.5: Tg=47+-0 #E+(2,2) #F+(7,5) #G+(25,11)
S=83(85,81) Mp= 969.5(0.0) Tp= 48 A2= 1/19 P=MAPQALLLL genpept PR=>gi|1780998|emb|CAA71531.1| (Y10520) HLA-C alpha chain (Cw*1701) [Homo sapiens] POS=4 (SEQ ID NO:98)

G=2489: Mg= 971.5: Tg=42+-0 #D+(9,4) #F+(10,6)
S=88(91,81) Mp= 971.4(-0.1) Tp= 42 A2= 1/23 P=FSNGYLASL genpept PR=>gi|12655065|gb|AAH01382.1|AAH01382 (BC001382) solute carrier family 29 (nucleoside transporters), member 1 [Homo sapiens] POS=405 (SEQ ID NO:99)

G=2495: Mg= 972.4: Tg=52+-1 #D+(25,5) #E+(19,9) #F+(24,7) #EST+(5,2) #FR+(8,3) #G+(43,11) #K+(32,6) #L+(4,2)
S=91(96,81) Mp= 972.5(0.1) Tp= 41 A2=656/30 P=TLIEDILGV genpept PR=>gi|11121497|emb|CAC14946.1| (AL132825) dJ756N5.2 (novel protein (DKFZp727M231) similar to Trp4-associated protein TAP1 (ABCB2)) [Homo sapiens] POS=209 (SEQ ID NO:100)

G=2514: Mg= 973.4: Tg=33+-1 #F+(5,3) #G+(10,6) #K+(4,2) #L+(1,1)
S=82(84,79) Mp= 973.4(0.0) Tp= 31 A2=0.0/17 P=IAEAVRTTL genpept PR=>gi|2559010|gb|AAC96011.1| (AF026292) chaperonin containing t-complex polypeptide 1, eta subunit; CCT-eta [Homo sapiens] POS=32 (SEQ ID NO:101)

G=2515: Mg= 973.5: Tg=34+-1 #EST+(5,2)
S=80(80,81) Mp= 973.4(-0.1) Tp= 31 A2=307/27 P=KLSELEAAL genpept PR=>gi|12314174|emb|CAC08001.1| (AL137067) bA13B9.3 (novel protein similar to KRT8) [Homo sapiens] POS=368 (SEQ ID NO:102)

G=2522: Mg= 974.3: Tg=30+-2 #S+(7,1) #E+(3,3) #EST+(8,2) #FR+(11,3)
S=89(90,89) Mp= 974.5(0.2) Tp= 25 A2= 6/21 P=SLSVKLEQA genpept PR=>gi|37258|emb|CAA44819.1| (X63105) Tpr [Homo sapiens] POS=453 (SEQ ID NO:104)

G=2527: Mg= 974.3: Tg=50+-0 #D+(1,1) #E+(15,7) #F+(9,4) #G+(22,10) #K+(10,5)
S=90(98,72) Mp= 974.5(0.2) Tp= 50 A2=413/26 P=MLLAALMIV genpept PR=>gi|5802822|gb|AAD51798.1|AF164614_2 (AF164614) envelope protein [Homo sapiens] POS=76 (SEQ ID NO:105)

G=2537: Mg= 974.5: Tg=53+-0 #F+(4,3) #G+(13,9) #K+(2,2)
S=81(83,79) Mp= 974.5(0.0) Tp= 56 A2= 60/24 P=AILPTSIFL
genpept PR=>gi|2323410|gb|AAB66581.1| (AF015913) Skb1Hs [Homo sapiens] POS=229 (SEQ ID NO:106)

G=2546: Mg= 975.4: Tg=38+-1 #E+(3,2) #F+(7,4) #G+(19,10) #K+(1,1)
S=82(91,63) Mp= 975.4(0.0) Tp= 32 A2= 8/27 P=AALPNVYEV
genpept PR=>gi|12652781|gb|AAH00142.1|AAH00142 (BC000142) minichromosome maintenance deficient (S. cerevisiae) 5 (cell division cycle 46) [Homo sapiens] POS=326 (SEQ ID NO:107)

G=2567: Mg= 977.5: Tg=22+-3 #G+(9,5)
S=84(82,90) Mp= 977.4(-0.1) Tp= 24 A2=186/24 P=RMLPHAPGV
genpept PR=>gi|1667394|gb|AAC50814.1| (U31814) transcriptional regulator homolog RPD3 [Homo sapiens] POS=372 (SEQ ID NO:108)

G=2610: Mg= 981.7: Tg=36+-0 #S #F+(3,2)
S=79(80,79) Mp= 981.6(-0.1) Tp= 38 A2= 49/32 P=SLIGHLQTL
genpept PR=>gi|642013|gb|AAB06261.1| (U16996) protein tyrosine posphatase [Homo sapiens] POS=337 (SEQ ID NO:10)

G=2636: Mg= 984.5: Tg=61+-1 #D+(5,4) #E+(9,5) #F+(12,6) #FR+(5,2) #G+(2,1) #K+(12,5) #L+(1,1)
S=85(91,72) Mp= 984.7(0.2) Tp= 61 A2= 11/21 P=LMVLVALIL
genpept PR=>gi|12654925|gb|AAH01309.1|AAH01309 (BC001309) Unknown (protein for MGC:5508) [Homo sapiens] POS=19 (SEQ ID NO:109)

G=2641: Mg= 984.7: Tg=36+-0 #EST+(1,1) #FR+(2,1)
S=78(77,81) Mp= 983.5(-1.2) Tp= 35 A2=140/28 P=KILPTLEAV
genpept PR=>gi|12653227|gb|AAH00382.1|AAH00382 (BC000382) interleukin enhancer binding factor 2, 45kD [Homo sapiens] POS=127 (SEQ ID NO:110)

G=2649: Mg= 985.5: Tg=40+-1 #E+(5,3) #FR+(3,2) #G+(4,3)
S=84(93,63) Mp= 985.6(0.1) Tp= 38 A2=>1k/33 P=ALLDRIVSV
genpept PR=>gi|1504030|dbj|BAA13214.1| (D86978) similar to a C.elegans protein encoded in cosmid K12D12(Z49069) [Homo sapiens] POS=1499 (SEQ ID NO:111)

G=2661: Mg= 986.6: Tg=35+-1 #E+(3,2) #F+(3,2) #EST+(3,2) #FR+(1,1) #G+(2,2)
S=84(82,89) Mp= 986.7(0.1) Tp= 35 A2=160/26 P=TLVYHVVGV
genpept PR=>gi|3540219|dbj|BAA32662.1| (D87686) KIAA0017 protein [Homo sapiens] POS=165 (SEQ ID NO:112)

G=2666: Mg= 987.4: Tg=32+-2 #D+(1,1) #E+(1,1) #F+(5,2) #G+(12,7)
S=77(87,54) Mp= 987.5(0.1) Tp= 33 A2=131/26 P=YLPPATQVV
genpept PR=>gi|13325146|gb|AAH04386.1|AAH04386 (BC004386) KIAA0111 gene product [Homo sapiens] POS=207 (SEQ ID NO:113)

G=2668: Mg= 987.4: Tg=14+-13 #F+(4,3)
S=77(76,81) Mp= 987.3(-0.1) Tp= 26 A2=0.0/15 P=PMEALAEQV
genpept PR=>gi|3882297|dbj|BAA34508.1| (AB018331) KIAA0788 protein [Homo sapiens] POS=569 (SEQ ID NO:114)

G=2671: Mg= 987.6: Tg=29+-1 #F+(4,3) #EST+(1,1) #FR+(3,2) #G+(11,5)

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S=74(83,54) Mp= 987.5(-0.1) Tp= 33 A2=656/30 P=RLSEAIVTV
genpept PR=>gi|7106848|gb|AAF36149.1|AF151063_1 (AF151063) HSPC229
[Homo sapiens] POS=137 (SEQ ID NO:115)

G=2677: Mg= 988.3: Tg=13+-4 #E+(2,1) #F+(7,1) #EST+(4,1) #G+(21,6)
S=88(99,63) Mp= 988.4(0.1) Tp= 20 A2= 28/27 P=SLDQPTQTV
genpept PR=>gi|1718197|gb|AAD03462.1| (U46025) translation intiation
factor eIF-3 p110 subunit [Homo sapiens] POS=834 (SEQ ID NO:116)

G=2692: Mg= 989.4: Tg=41+-1 #S+(8,2) #D+(13,5) #E+(12,6) #F+(11,6)
#EST+(4,2) #FR+(6,3) #G+(15,8) #K+(13,6)
S=79(83,72) Mp= 989.5(0.1) Tp= 39 A2=257/30 P=SLFPGKLEV
genpept PR=>gi|440177|gb|AAC03568.1| (U01184) flightless-I homolog
[Homo sapiens] POS=1009 (SEQ ID NO:12)

G=2693: Mg= 989.5: Tg=31+-2 #S+(15,2) #E+(7,3) #F+(13,5)
#EST+(3,1) #G+(12,7) #K+(6,4)
S=83(84,81) Mp= 989.5(0.0) Tp= 35 A2= 88/29 P=SLSEKTVLL
genpept PR=>gi|180151|gb|AAA88793.1| (M84349) CD59 protein [Homo
sapiens] POS=106 (SEQ ID NO:11)

G=2729: Mg= 993.5: Tg=18+-4 #F+(2,1) #EST+(4,2) #FR+(8,3) #G+(9,4)
#K+(1,1)
S=92(97,81) Mp= 993.6(0.1) Tp= 22 A2=243/23 P=KLHGVNINV
genpept PR=>gi|12653083|gb|AAH00307.1|AAH00307 (BC000307) RNA
binding motif protein 4 [Homo sapiens] POS=59 (SEQ ID NO:117)

G=2769: Mg= 999.5: Tg=35+-1 #H+(5,3) #I+(8,4) #J+(5,4)
S=82(83,81) Mp= 999.5(0.0) Tp= 39 A2= 5/18 P=LVMAPRTVL
genpept PR=>gi|9738918|gb|AAF97847.1| (AF129293) MHC class I antigen
[Homo sapiens] POS=2 (SEQ ID NO:118)

G=2773: Mg= 999.6: Tg=45+-1 #D+(2,1) #E+(15,6) #F+(12,7)
#EST+(3,1) #FR+(8,3) #G+(15,8) #K+(11,5) #L+(1,1)
S=80(86,69) Mp= 999.6(0.0) Tp= 42 A2= 22/31 P=SIIGRLLEV
genpept PR=>gi|190516|gb|AAA36508.1| (M63960) protein phosphatase-1
[Homo sapiens] POS=11 (SEQ ID NO:119)

G=2785: Mg=1000.5: Tg=33+-1 #G+(14,6) #K+(2,2)
S=77(77,79) Mp=1000.6(0.1) Tp= 36 A2= 2/16 P=MAVALQLRV
genpept PR=>gi|11544742|emb|CAC17582.1| (AL121997) dJ1043F6.1.1
(Chediak-Higashi syndrome 1 (isoform 1)) [Homo sapiens] POS=2544
(SEQ ID NO:120)

G=2789: Mg=1000.6: Tg=26+-2 #F+(3,2) #EST+(2,2) #FR+(2,1)
#G+(13,6) #K+(1,1)
S=90(90,90) Mp=1000.4(-0.2) Tp= 27 A2=656/30 P=GLNEEIARV
genpept PR=>gi|2501873|gb|AAB80726.1| (AF017790)
retinoblastoma-associated protein HEC [Homo sapiens] POS=330 (SEQ
ID NO:121)

G=2791: Mg=1001.3: Tg=40+-1 #F+(10,5) #G+(16,9) #K+(4,3)
S=78(81,72) Mp=1001.6(0.3) Tp= 19 A2=0.9/23 P=IMKVAQAKL
genpept PR=>gi|6941888|gb|AAF32263.1|AF170562_1 (AF170562)
ubiquitin-specific processing protease [Homo sapiens] POS=875 (SEQ
ID NO:122)

G=2822: Mg=1004.2: Tg=27+-1 #G+(8,5)
S=90(91,90) Mp=1004.4(0.2) Tp= 30 A2= 88/25 P=TLSEVTNQL
genpept PR=>gi|12053045|emb|CAB66698.1| (AL136764) hypothetical protein [Homo sapiens] POS=484 (SEQ ID NO:123)

G=2829: Mg=1004.5: Tg=38+-1 #F+(3,2) #EST+(1,1) #FR+(2,1) #G+(9,6)
S=91(92,90) Mp=1004.6(0.1) Tp= 37 A2=324/29 P=ALFEGKVQL
genpept PR=>gi|10439712|dbj|BAB15550.1| (AK026780) unnamed protein product [Homo sapiens] POS=442 (SEQ ID NO:124)

G=2833: Mg=1004.6: Tg=29+-0 #EST+(3,1)
S=87(87,89) Mp=1004.6(0.0) Tp= 31 A2= 32/28 P=GLKGRVFEV
genpept PR=>gi|854179|emb|CAA60827.1| (X87373) ribosomal protein S3a [Homo sapiens] POS=61 (SEQ ID NO:125)

G=2835: Mg=1005.2: Tg=48+-0 #G+(3,3)
S=84(83,89) Mp=1005.5(0.3) Tp= 42 A2= 35/25 P=NIFPYPVGV
genpept PR=>gi|2822460|gb|AAC39565.1| (AF030234) splicing factor Sip1 [Homo sapiens] POS=912 (SEQ ID NO:126)

G=2872: Mg=1009.6: Tg=47+-1 #E+(2,1) #EST+(5,2) #FR+(6,3) #K+(15,6)
S=87(96,66) Mp=1009.7(0.1) Tp= 52 A2= 3/18 P=LVSIVVAVPL
genpept PR=>gi|7023136|dbj|BAA91851.1| (AK001708) unnamed protein product [Homo sapiens] POS=23 (SEQ ID NO:127)

G=2881: Mg=1010.5: Tg=28+-1 #G+(8,5)
S=84(85,82) Mp=1010.5(0.0) Tp= 20 A2=370/30 P=NMYGKVVTV
genpept PR=>gi|1845267|gb|AAC51102.1| (U56402) SUPT5H [Homo sapiens] POS=562 (SEQ ID NO:128)

G=2891: Mg=1011.5: Tg=43+-1 #E+(9,5) #F+(1,1) #EST+(3,2) #FR+(6,3) #G+(13,7) #K+(5,3) #L+(3,2)
S=79(78,82) Mp=1011.5(0.0) Tp= 45 A2=>1k/31 P=LLLDVPTAAV
genpept PR=>gi|6165618|gb|AAF04618.1|AF097362_1 (AF097362) gamma-interferon inducible lysosomal thiol reductase [Homo sapiens] POS=26 (SEQ ID NO:2) ref

G=2918: Mg=1014.4: Tg=48+-0 #D+(1,1) #E+(16,8) #F+(11,7) #EST+(2,1) #FR+(3,2) #G+(19,10)
S=88(97,68) Mp=1014.6(0.2) Tp= 46 A2=160/32 P=SLINVGLISV
genpept PR=>gi|12653413|gb|AAH00476.1|AAH00476 (BC000476) acidic protein rich in leucines [Homo sapiens] POS=48 (SEQ ID NO:129)

G=2928: Mg=1015.4: Tg=56+-0 #E+(26,8) #EST+(2,1) #FR+(5,3)
S=92(97,81) Mp=1015.5(0.1) Tp= 61 A2=666/30 P=ALLGTLWEI
genpept PR=>gi|2224595|dbj|BAA20785.1| (AB002325) KIAA0327 protein [Homo sapiens] POS=18 (SEQ ID NO:130)

G=2929: Mg=1015.4: Tg=41+-1 #E+(5,3) #EST+(4,2) #FR+(2,2) #G+(12,7) #K+(5,3)
S=81(86,72) Mp=1015.5(0.1) Tp= 39 A2= 13/16 P=FQDPVPLTV
genpept PR=>gi|4325107|gb|AAD17258.1| (AF119042) transcriptional intermediary factor 1 alpha; TIF1alpha [Homo sapiens] POS=890 (SEQ ID NO:131)

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G=2947: Mg=1016.4: Tg=45+-1 #E+(3,3) #F+(8,5) #EST+(2,2) #FR+(3,1) #G+(18,10) #K+(7,4)
S=82(95,54) Mp=1016.6(0.2) Tp= 39 A2=512/28 P=GLYPNLIQV
genpept PR=>gi|4240269|dbj|BAA74913.1| (AB020697) KIAA0890 protein [Homo sapiens] POS=1022 (SEQ ID NO:132)

G=2965: Mg=1018.4: Tg=23+-4 #D+(3,1) #E+(2,2) #F+(2,2) #G+(25,8)
S=94(96,90) Mp=1018.6(0.2) Tp= 19 A2= 79/26 P=VMDSKIVQV
genpept PR=>gi|913393|gb|AAC60648.1| (S75295) nucleoprotein interactor 1, NPI-1=SRP1 homolog [human, cervical carcinoma HeLa cells, Peptide, 538 aa] [Homo sapiens] POS=434 (SEQ ID NO:133)

G=2976: Mg=1019.6: Tg=46+-0 #D+(5,2) #E+(6,1) #F+(2,2) #EST+(1,1) #FR+(2,1) #G+(4,3)
S=83(81,90) Mp=1019.6(0.0) Tp= 40 A2=745/32 P=ALLDKLYAL
genpept PR=>gi|7023341|dbj|BAA91929.1| (AK001830) unnamed protein product [Homo sapiens] POS=78 (SEQ ID NO:134)

G=2985: Mg=1020.5: Tg=45+-0 #D+(5,3) #E+(3,2) #F+(4,3) #FR+(1,1) #G+(7,5)
S=99(99,99) Mp=1020.5(0.0) Tp= 40 A2=298/27 P=NLASFIEQV
genpept PR=>gi|348907|gb|AAA35672.1| (L15428) 4a-carbinolamine dehydratase [Homo sapiens] POS=19 (SEQ ID NO:135)

G=2998: Mg=1022.4: Tg=44+-0 #G+(5,3)
S=76(70,90) Mp=1022.4(0.0) Tp= 43 A2=0.7/12 P=TLWVDPYE
genpept PR=>gi|1703501|gb|AAB37580.1| (U72649) BTG2 [Homo sapiens] POS=101 (SEQ ID NO:136)

G=3002: Mg=1022.5: Tg=45+-1 #S+(2,1) #D+(3,2) #G+(7,4)
S=82(83,81) Mp=1022.5(0.0) Tp= 42 A2=>1k/25 P=KIADFGWSV
genpept PR=>gi|3127068|gb|AAC77369.1| (AF059681) serine/threonine kinase 13 [Homo sapiens] POS=147 (SEQ ID NO:137)

G=3036: Mg=1025.5: Tg=37+-1 #S #D+(1,1) #F+(4,2) #EST+(1,1) #G+(5,3)
S=90(91,90) Mp=1025.6(0.1) Tp= 36 A2= 89/28 P=SLLSHVEQL
genpept PR=>gi|5305429|gb|AAD41647.1|AF072933_1 (AF072933) Mad2-like protein [Homo sapiens] POS=114 (SEQ ID NO:138)

G=3041: Mg=1026.3: Tg=45+-0 #D+(7,3) #FR+(1,1) #G+(4,3)
S=84(90,72) Mp=1025.6(-0.7) Tp= 38 A2=>1k/30 P=GLADKVYFL
genpept PR=>gi|1228049|dbj|BAA11423.1| (D78586) multifunctional protein CAD [Homo sapiens] POS=445 (SEQ ID NO:139)

G=3061: Mg=1028.5: Tg=35+-1 #S+(6,2) #D+(3,1) #E+(20,7) #F+(8,5) #EST+(5,2) #FR+(5,2) #G+(11,7)
S=88(92,81) Mp=1028.5(0.0) Tp= 32 A2= 88/28 P=GLIEKNIEL
genpept PR=>gi|1632819|emb|CAA45219.1| (X63692) DNA (cytosine-5-)-methyltransferase [Homo sapiens] POS=425 (SEQ ID NO:13)

G=3073: Mg=1029.5: Tg=51+-0 #D+(1,1) #FR+(2,1) #G+(5,4) #K+(2,1)
S=81(78,90) Mp=1029.6(0.1) Tp= 35 A2=>1k/31 P=SLLDIIEKV
genpept PR=>gi|1063586|gb|AAB41564.1| (L48546) tuberin [Homo sapiens] POS=526 (SEQ ID NO:140)

G=3092: Mg=1031.4: Tg=61+-1 #S+(8,2) #D+(29,4) #E+(10,6) #F+(3,1) #EST+(2,1) #FR+(5,2) #H+(1,1) #K+(1,1)
S=84(82,90) Mp=1031.6(0.2) Tp= 64 A2=865/30 P=GLYPGLIWL
genpept PR=>gi|2599385|gb|AAB84111.1| (AF027292) interferon regulatory factor 6 [Homo sapiens] POS=21 (SEQ ID NO:14)

G=3118: Mg=1034.4: Tg=60+-1 #D+(16,5) #E+(45,9) #F+(14,7) #EST+(2,1) #FR+(10,3) #G+(11,7) #K+(7,4)
S=81(85,72) Mp=1034.6(0.2) Tp= 66 A2= 32/21 P=FVFPGELLL
genpept PR=>gi|12652633|gb|AAH00062.1|AAH00062 (BC000062) solute carrier family 1 (neutral amino acid transporter), member 5 [Homo sapiens] POS=89 (SEQ ID NO:141)

G=3127: Mg=1036.3: Tg=36+-0 #F+(2,1)
S=78(77,81) Mp=1036.6(0.3) Tp= 35 A2=656/30 P=ALNELLQHV
genpept PR=>gi|6682361|gb|AAF23322.1|AF177198_1 (AF177198) talin [Homo sapiens] POS=777 (SEQ ID NO:142)ref

G=3128: Mg=1036.3: Tg=36+-1 #G+(12,7)
S=83(84,81) Mp=1036.5(0.2) Tp= 29 A2=913/27 P=NLYEGQITV
genpept PR=>gi|1699038|gb|AAC50967.1| (U78735) ABC3 [Homo sapiens] POS=555 (SEQ ID NO:143)

G=3142: Mg=1037.5: Tg=43+-1 #EST+(2,2) #FR+(5,2) #G+(1,1) #I+(11,5) #J+(5,3)
S=86(89,79) Mp=1037.5(0.0) Tp= 41 A2=0.1/15 P=FTKDFAPVI
genpept PR=>gi|7022824|dbj|BAA91736.1| (AK001518) unnamed protein product [Homo sapiens] POS=77 (SEQ ID NO:144)

G=3144: Mg=1037.6: Tg=51+-1 #D+(7,3) #E+(29,6) #F+(11,7) #EST+(2,1) #FR+(4,2) #G+(12,7) #K+(3,2) #L+(1,1)
S=87(86,90) Mp=1037.7(0.1) Tp= 53 A2=>1k/31 P=KLLEPVLLL
genpept PR=>gi|338447|gb|AAA60583.1| (M60854) RPS16 [Homo sapiens] POS=50 (SEQ ID NO:145)ref

G=3154: Mg=1038.5: Tg=48+-1 #D+(32,4) #E+(48,9) #F+(7,5) #EST+(6,2) #FR+(9,3) #G+(24,10) #K+(9,6)
S=81(82,81) Mp=1038.7(0.2) Tp= 47 A2=408/30 P=YLLPAIVHI
genpept PR=>gi|2832596|emb|CAB09792.1| (Z97056) dJ434P1.3 (DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 17 (72kD)) [Homo sapiens] POS=146 (SEQ ID NO:15) ref

G=3183: Mg=1041.4: Tg=52+-0 #FR+(2,1) #G+(1,1)
S=79(82,72) Mp=1041.6(0.2) Tp= 52 A2=>1k/23 P=GLFAPQFYV
genpept PR=>gi|2062371|gb|AAB65850.1| (U70730) SnoN2 [Homo sapiens] POS=274 (SEQ ID NO:146)

G=3191: Mg=1042.4: Tg=29+-1 #S+(2,1) #G+(12,6)
S=87(90,81) Mp=1042.5(0.1) Tp= 27 A2=805/27 P=LMVDHVTEV
genpept PR=>gi|9930612|gb|AAG02115.1|AF293025_1 (AF293025) steroid receptor RNA activator isoform 2 [Homo sapiens] POS=183 (SEQ ID NO:147)

G=3201: Mg=1043.5: Tg=58+-1 #E+(2,2) #F+(6,4) #EST+(1,1) #FR+(2,1) #G+(7,4) #K+(1,1)

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S=85(88,81) Mp=1043.7(0.2) Tp= 62 A2=408/27 P=FLLPILSQI genpept PR=>gi|2580552|gb|AAC51830.1| (AF000983) dead box, X isoform [Homo sapiens] POS=234 (SEQ ID NO:148)

G=3213: Mg=1045.5: Tg=58+-0 #E+(1,1) #F+(2,2) #FR+(1,1)
S=84(90,72) Mp=1044.5(-1.0) Tp= 54 A2=0.3/18 P=FLIPLNITN genpept PR=>gi|2224611|dbj|BAA20793.1| (AB002333) KIAA0335 [Homo sapiens] POS=938 (SEQ ID NO:149)

G=3219: Mg=1046.6: Tg=40+-1 #D+(2,2) #E+(1,1) #F+(2,1) #EST+(4,1) #FR+(5,2) #G+(1,1) #K+(3,3)
S=85(83,90) Mp=1046.7(0.1) Tp= 39 A2=243/30 P=NLLPKLHIV genpept PR=>gi|4588524|gb|AAD26136.1|AF109196_1 (AF109196) intracellular chloride channel p64H1 [Homo sapiens] POS=190 (SEQ ID NO:150)

G=3227: Mg=1047.6: Tg=44+-0 #D+(1,1) #E+(15,8) #F+(2,2) #EST+(5,2) #FR+(7,3) #G+(12,6)
S=79(82,72) Mp=1047.6(0.0) Tp= 50 A2=413/31 P=LLDRFLATV genpept PR=>gi|12653303|gb|AAH00420.1|AAH00420 (BC000420) cyclin I [Homo sapiens] POS=72 (SEQ ID NO:151)

G=3240: Mg=1049.4: Tg=41+-1 #E+(3,2) #F+(2,2) #EST+(2,2) #FR+(3,3)
S=77(79,74) Mp=1049.5(0.1) Tp= 36 A2=294/29 P=YLDPSVLSGV genpept PR=>gi|505098|dbj|BAA06683.1| (D31885) KIAA0069 [Homo sapiens] POS=84 (SEQ ID NO:152)

G=3242: Mg=1049.5: Tg=44+-0 #F+(8,6)
S=78(85,63) Mp=1048.5(-1.0) Tp= 44 A2=378/27 P=LLYPTEITV genpept PR=>gi|220141|dbj|BAA00845.1| (D01038) VLA-3 alpha subunit [Homo sapiens] POS=798 (SEQ ID NO:153)

G=3257: Mg=1051.4: Tg=65+-1 #D+(2,2) #E+(9,4) #F+(9,4) #EST+(2,1) #FR+(6,3)
S=88(88,90) Mp=1051.6(0.2) Tp= 63 A2=>1k/26 P=NLGDFLIFL genpept PR=>gi|1469175|dbj|BAA09475.1| (D50916) The KIAA0126 gene is partially related to a yeast gene. [Homo sapiens] POS=638 (SEQ ID NO:154)

G=3258: Mg=1051.4: Tg=54+-0 #D+(18,4) #E+(10,6) #F+(8,5) #G+(1,1) #K+(3,2)
S=79(85,66) Mp=1051.5(0.1) Tp= 56 A2=>1k/30 P=GLYEGLTWL genpept PR=>gi|178989|gb|AAA90928.1| (M57763) ADP-ribosylation factor [Homo sapiens] POS=161 (SEQ ID NO:155)

G=3270: Mg=1054.3: Tg=51+-0 #D+(5,3) #E+(19,8) #F+(12,7) #EST+(2,1) #FR+(5,2)
S=96(96,99) Mp=1054.5(0.2) Tp= 48 A2=437/19 P=SLFDLNFQA genpept PR=>gi|189292|gb|AAB60701.1| (M81600) NAD(P)H:quinone oxireductase [Homo sapiens] POS=227 (SEQ ID NO:156)

G=3271: Mg=1054.3: Tg=55+-1 #K+(5,2)
S=80(77,90) Mp=1054.4(0.1) Tp= 43 A2=0.0/8 P=MFSLEDSII genpept PR=>gi|809029|emb|CAA57993.1| (X82676) tyrosine phosphatase [Homo sapiens] POS=833 (SEQ ID NO:157)

G=3279: Mg=1055.4: Tg=37+-1 #G+(6,4)

S=76(74,81) Mp=1055.3(-0.1) Tp= 37 A2=122/19 P=AMWEHPITA
genpept PR=>gi|10197638|gb|AAG14955.1|AF182419_1 (AF182419) MDS018 [Homo sapiens] POS=65 (SEQ ID NO:158)

G=3297: Mg=1057.5: Tg=17+-2 #G+(8,4)
S=95(94,99) Mp=1057.6(0.1) Tp= 31 A2=320/26 P=YLGRLAHEV
genpept PR=>gi|12653485|gb|AAH00514.1|AAH00514 (BC000514) ribosomal protein L13a [Homo sapiens] POS=137 (SEQ ID NO:159)

G=3309: Mg=1059.5: Tg=34+-0 #F+(3,3) #EST+(3,1) #FR+(2,1)
S=82(84,79) Mp=1059.6(0.1) Tp= 30 A2=482/24 P=GLIDHQTYL
genpept PR=>gi|1477651|gb|AAB05428.1| (U63610) plectin [Homo sapiens] POS=4188 (SEQ ID NO:160)

G=3325: Mg=1061.4: Tg=40+-1 #G+(6,3)
S=85(87,81) Mp=1061.7(0.3) Tp= 31 A2=523/26 P=AIQDKLFQV
genpept PR=>gi|13543970|gb|AAH06123.1|AAH06123 (BC006123) Similar to RIKEN cDNA 0710001P09 gene [Homo sapiens] POS=96 (SEQ ID NO:161)

G=3329: Mg=1062.4: Tg=29+-0 #H+(6,3)
S=89(89,89) Mp=1062.5(0.1) Tp= 27 A2=0.0/9 P=IVKWDRDM
genpept PR=>gi|179318|gb|AAA51811.1| (M17987) beta-2-microglobulin [Homo sapiens] POS=112 (SEQ ID NO:162)

G=3331: Mg=1062.5: Tg=33+-1 #F+(9,6) #EST+(1,1) #G+(18,10) #K+(4,3)
S=86(97,63) Mp=1062.6(0.1) Tp= 30 A2= 6/20 P=RIIDVVYNA
genpept PR=>gi|36150|emb|CAA47670.1| (X67247) ribosomal protein S8 [Homo sapiens] POS=77 (SEQ ID NO:163)

G=3342: Mg=1064.4: Tg=20+-4 #E+(1,1) #F+(6,4) #EST+(3,1) #G+(16,6)
S=86(88,82) Mp=1064.6(0.2) Tp= 19 A2=439/28 P=KIYEGQVEV
genpept PR=>gi|550013|gb|AAA85654.1| (U14966) ribosomal protein L5 [Homo sapiens] POS=117 (SEQ ID NO:164)

G=3364: Mg=1066.3: Tg=50+-0 #E+(3,2) #G+(18,10) #K+(9,5) #L+(1,1)
S=81(85,72) Mp=1066.6(0.3) Tp= 50 A2=736/26 P=FLPSYIIDV
genpept PR=>gi|1045574|gb|AAC50293.1| (U37012) cleavage and polyadenylation specificity factor [Homo sapiens] POS=185 (SEQ ID NO:165)

G=3384: Mg=1068.4: Tg=29+-2 #S+(8,2) #D+(3,1) #E+(17,4) #F+(8,5) #EST+(5,2) #FR+(6,3) #G+(18,7) #K+(4,2)
S=87(90,81) Mp=1068.6(0.2) Tp= 29 A2=482/23 P=ALSDHHIYL
genpept PR=>gi|28597|emb|CAA28861.1| (X05236) aldolase A (AA 1-364) [Homo sapiens] POS=216 (SEQ ID NO:16) ref

G=3385: Mg=1069.3: Tg=37+-1 #F+(2,2) #G+(7,5)
S=84(92,66) Mp=1069.3(0.0) Tp= 25 A2=855/25 P=YMMPVNSEV
genpept PR=>gi|12667401|gb|AAK01426.1|AF326731_1 (AF326731) NUF2R [Homo sapiens] POS=65 (SEQ ID NO:166)

G=3406: Mg=1071.5: Tg=20+-3 #D+(1,1) #FR+(2,1) #G+(20,7)
S=94(97,90) Mp=1071.6(0.1) Tp= 24 A2=109/30 P=ILDQKINEV
genpept PR=>gi|338278|gb|AAA60563.1| (M31061) ornithine decarboxylase [Homo sapiens] POS=23 (SEQ ID NO:17) ref

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G=3410: Mg=1071.6: Tg=8+-8 #D+(7,1) #F+(6,1) #FR+(6,1) #G+(25,5) #K+(4,3)
S=94(96,90) Mp=1071.7(0.1) Tp= 12 A2= 53/29 P=ILDKKVEKV
genpept PR=>gi|386786|gb|AAA36026.1| (J04988) 90 kD heat shock protein [Homo sapiens] POS=570 (SEQ ID NO:18) ref

G=3418: Mg=1073.6: Tg=5+-7 #F+(1,1) #G+(3,3)
S=79(79,81) Mp=1072.5(-1.1) Tp= 5 A2=0.1/14 P=NKDLKMPKV
genpept PR=>gi|1808578|dbj|BAA07918.1| (D44466) proteasome subunit p112 [Homo sapiens] POS=792 (SEQ ID NO:167)

G=3424: Mg=1074.6: Tg=46+-0 #K+(6,4)
S=90(90,90) Mp=1074.4(-0.2) Tp= 31 A2=201/28 P=NLAEDIMRL
genpept PR=>gi|37852|emb|CAA79613.1| (Z19554) vimentin [Homo sapiens] POS=177 (SEQ ID NO:168)

G=3427: Mg=1075.4: Tg=46+-0 #D+(1,1) #F+(8,6)
S=72(73,72) Mp=1075.6(0.2) Tp= 44 A2=>1k/31 P=YLPELLQTV
genpept PR=>gi|12653299|gb|AAH00418.1|AAH00418 (BC000418) ectodermal-neural cortex (with BTB-like domain) [Homo sapiens] POS=228 (SEQ ID NO:169)

G=3470: Mg=1080.4: Tg=62+-1 #D+(13,4) #E+(17,8) #F+(11,6) #EST+(2,1) #FR+(6,3) #G+(19,9) #K+(10,5) #L+(1,1)
S=82(82,82) Mp=1080.6(0.2) Tp= 69 A2=>1k/27 P=FLYPFPLAL
genpept PR=>gi|436224|dbj|BAA05062.1| (D26067) KIAA0033 [Homo sapiens] POS=185 (SEQ ID NO:170)

G=3472: Mg=1080.4: Tg=50+-0 #F+(13,7) #G+(25,11) #K+(10,5)
S=76(87,52) Mp=1080.5(0.1) Tp= 53 A2=182/33 P=SLLPPTALVGL
genpept PR=>gi|1296664|emb|CAA65774.1| (X97064) Sec23 protein [Homo sapiens] POS=156 (SEQ ID NO:19)

G=3476: Mg=1080.7: Tg=41+-1 #FR+(1,1) #G+(5,4)
S=75(77,72) Mp=1080.6(-0.1) Tp= 38 A2=>1k/29 P=NLYPFVKTV
genpept PR=>gi|1263196|gb|AAA97405.1| (U37436) AICAR formyltransferase/IMP cyclohydrolase bifunctional enzyme [Homo sapiens] POS=101 (SEQ ID NO:171)

G=3477: Mg=1081.4: Tg=56+-0 #F+(6,3)
S=90(87,99) Mp=1081.7(0.3) Tp= 57 A2=>1k/24 P=SVIEQLFFV
genpept PR=>gi|30140|emb|CAA34277.1| (X16155) COUP-TF [Homo sapiens] POS=378 (SEQ ID NO:172)

G=3478: Mg=1081.4: Tg=56+-0 #G+(4,3)
S=84(86,81) Mp=1080.6(-0.8) Tp= 57 A2=>1k/29 P=SLLEPFVYL
genpept PR=>gi|7008404|gb|AAF34999.1| (AF229840) kappa B-ras 2 [Homo sapiens] POS=156 (SEQ ID NO:173)

G=3497: Mg=1084.7: Tg=24+-3 #F+(1,1) #EST+(3,1) #FR+(2,1)
S=79(82,72) Mp=1084.6(-0.1) Tp= 37 A2=437/26 P=ILFGHENRV
genpept PR=>gi|5911941|emb|CAB55946.1| (AL117471) hypothetical protein [Homo sapiens] POS=250 (SEQ ID NO:174)ref

G=3505: Mg=1086.5: Tg=19+-3 #G+(18,6)

S=88(91,83) Mp=1086.7(0.2) Tp= 20 A2=998/29 P=KLQEVGQVSV
genpept PR=>gi|340307|gb|AAA36808.1| (M14648) vitronectin alpha subunit precursor [Homo sapiens] POS=338 (SEQ ID NO:175)

G=3520: Mg=1088.6: Tg=37+-1 #F+(3,2) #EST+(2,1) #G+(8,5) #K+(5,4)
S=72(82,49) Mp=1088.5(-0.1) Tp= 37 A2= 75/16 P=RLFDEPQLA
genpept PR=>gi|3334982|gb|AAC26984.1|AAC26984 (AC005306) R27216_1 [Homo sapiens] POS=2 (SEQ ID NO:176)

G=3521: Mg=1088.6: Tg=44+-1 #G+(5,4)
S=70(71,68) Mp=1088.6(0.0) Tp= 43 A2=119/30 P=SLFPGKLEVV
genpept PR=>gi|440177|gb|AAC03568.1| (U01184) flightless-I homolog [Homo sapiens] POS=1009 (SEQ ID NO:177)

G=3526: Mg=1089.6: Tg=50+-1 #S+(10,1)
S=84(91,68) Mp=1089.6(0.0) Tp= 57 A2= 37/23 P=VMLGTPFLVI
genpept PR=>gi|4589536|dbj|BAA76790.1| (AB023163) KIAA0946 protein [Homo sapiens] POS=340 (SEQ ID NO:178)

G=3533: Mg=1091.4: Tg=15+-2 #G+(12,4)
S=86(95,68) Mp=1091.4(0.0) Tp= 13 A2= 80/20 P=GVYDGEEHSV
genpept PR=>gi|4102749|gb|AAD01565.1| (AF015766) MAGE XP-2 protein [Homo sapiens] POS=231 (SEQ ID NO:20)

G=3545: Mg=1094.4: Tg=50+-1 #E+(22,9) #F+(16,7) #EST+(4,2) #FR+(4,2) #G+(50,11) #K+(11,6) #L+(2,2)
S=80(89,59) Mp=1094.5(0.1) Tp= 49 A2=182/33 P=SLLPPDALVGL
genpept PR=>gi|13529299|gb|AAH05404.1|AAH05404 (BC005404) Unknown (protein for MGC:5020) [Homo sapiens] POS=156 (SEQ ID NO:21)

G=3563: Mg=1098.3: Tg=38+-1 #D+(7,3) #E+(10,6) #F+(5,4) #EST+(3,2) #FR+(4,2) #G+(12,8)
S=88(88,90) Mp=1098.4(0.1) Tp= 30 A2=280/26 P=SLYDYNPNL
genpept PR=>gi|3337383|gb|AAC27426.1| (AC002544) Translation initiation factor eIF-p110 [Homo sapiens] POS=381 (SEQ ID NO:179)

G=3566: Mg=1098.6: Tg=50+-0 #E+(6,3) #EST+(3,1) #FR+(2,1)
S=82(89,68) Mp=1098.7(0.1) Tp= 62 A2=194/25 P=FLLGPRLVLA
genpept PR=>gi|887368|gb|AAC42003.1| (L40397) ORF; putative [Homo sapiens] POS=31 (SEQ ID NO:180)

G=3579: Mg=1101.4: Tg=34+-1 #F+(5,4) #G+(2,2)
S=88(91,83) Mp=1101.4(0.0) Tp= 35 A2=502/24 P=FLYTGEGDTV
genpept PR=>gi|1184320|gb|AAC50373.1| (U45880) X-linked inhibitor of apotosis protein [Homo sapiens] POS=52 (SEQ ID NO:181)

G=3588: Mg=1102.6: Tg=34+-1 #E+(1,1) #EST+(4,2) #FR+(2,2)
S=82(83,81) Mp=1102.6(0.0) Tp= 27 A2=>1k/26 P=KLNPQQFEV
genpept PR=>gi|624704|gb|AAB05994.1| (L38961) putative transmembrane protein precursor [Homo sapiens] POS=289 (SEQ ID NO:182)

G=3596: Mg=1103.4: Tg=28+-2 #F+(3,2) #G+(1,1)
S=88(94,74) Mp=1103.4(0.0) Tp= 23 A2=140/27 P=SLADLQNDEV
genpept PR=>gi|854179|emb|CAA60827.1| (X87373) ribosomal protein S3a [Homo sapiens] POS=70 (SEQ ID NO:183)

G=3603: Mg=1104.7: Tg=45+-0 #D+(1,1) #F+(4,3) #EST+(3,1) #FR+(5,2)

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S=85(99,54) Mp=1104.7(0.0) Tp= 40 A2=364/28 P=RLLDYVVNI
genpept PR=>gi|7023768|dbj|BAA92081.1| (AK002094) unnamed protein product [Homo sapiens] POS=172 (SEQ ID NO:184)

G=3629: Mg=1113.5: Tg=35+-1 #G+(10,6) #K+(10,5)
S=77(76,81) Mp=1113.6(0.1) Tp= 31 A2= 46/21 P=FVDDYTVRV
genpept PR=>gi|1923256|gb|AAC51866.1| (U86782) 26S proteasome-associated pad1 homolog [Homo sapiens] POS=61 (SEQ ID NO:185)

G=3637: Mg=1115.4: Tg=55+-1 #E+(29,8) #F+(14,7) #EST+(2,1) #FR+(4,2) #G+(9,5) #L+(1,1)
S=82(90,66) Mp=1115.5(0.1) Tp= 61 A2=>1k/29 P=SLFEGTWYL
genpept PR=>gi|12653065|gb|AAH00297.1|AAH00297 (BC000297) 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble) [Homo sapiens] POS=447 (SEQ ID NO:186)

G=3652: Mg=1119.5: Tg=56+-0 #D+(4,3)
S=80(84,72) Mp=1119.7(0.2) Tp= 57 A2=512/27 P=ALYNWLIQV
genpept PR=>gi|3288447|emb|CAA07553.1| (AJ007558) nucleoporin 155 [Homo sapiens] POS=1038 (SEQ ID NO:187)

G=3653: Mg=1119.6: Tg=30+-1 #D+(1,1) #F+(3,2) #EST+(1,1) #FR+(2,1) #G+(10,5)
S=80(84,72) Mp=1119.7(0.1) Tp= 30 A2= 97/25 P=VLIDYQRNV
genpept PR=>gi|2626840|dbj|BAA23415.1| (D89729) CRM1 protein [Homo sapiens] POS=784 (SEQ ID NO:188)

G=3658: Mg=1121.3: Tg=49+-0 #S+(9,2) #D+(7,3) #E+(8,5) #F+(14,7) #G+(26,11) #K+(2,2) #L+(1,1)
S=84(81,91) Mp=1121.5(0.2) Tp= 47 A2=577/24 P=TLWVDPYEV
genpept PR=>gi|1703501|gb|AAB37580.1| (U72649) BTG2 [Homo sapiens] POS=101 (SEQ ID NO:22) ref

G=3683: Mg=1128.3: Tg=51+-0 #S #G+(17,9) #K+(2,1)
S=86(88,82) Mp=1128.5(0.2) Tp= 55 A2=348/25 P=FTWEGLYNV
genpept PR=>gi|1276912|gb|AAC50450.1| (U44839) UHX1 protein [Homo sapiens] POS=353 (SEQ ID NO:189)

G=3694: Mg=1133.6: Tg=25+-3 #D+(2,1) #F+(1,1) #G+(7,3)
S=85(87,81) Mp=1133.7(0.1) Tp= 30 A2=>1k/32 P=ILMEHIHKL
genpept PR=>gi|298486|gb|AAB25672.1| (S56985) ribosomal protein L19 [human, breast cancer cell line, MCF-7, Peptide, 196 aa] [Homo sapiens] POS=137 (SEQ ID NO:190) ref

G=3697: Mg=1134.6: Tg=42+-1 #E+(12,6) #F+(1,1) #EST+(3,1) #FR+(3,1) #G+(9,5) #K+(13,6) #L+(1,1)
S=81(93,53) Mp=1134.6(0.0) Tp= 37 A2=193/26 P=RLDELGGVYL
genpept PR=>gi|13374901|emb|CAC34517.1| (AL031659) dJ343K2.2.3 (ribophorin II (isoform 3)) [Homo sapiens] POS=185 (SEQ ID NO:191)

G=3711: Mg=1140.6: Tg=40+-1 #EST+(1,1) #FR+(1,1) #G+(1,1)
S=89(93,82) Mp=1140.7(0.1) Tp= 40 A2=526/27 P=KLLSKFYEL
genpept PR=>gi|10439903|dbj|BAB15591.1| (AK026930) unnamed protein product [Homo sapiens] POS=231 (SEQ ID NO:192)

G=3721: Mg=1145.4: Tg=49+-1 #S+(7,1) #F+(2,2) #G+(14,10)

S=79(83,70) Mp=1145.5(0.1) Tp= 50 A2=>1k/23 P=FLFDGSPTYV genpept PR=>gi|1049053|gb|AAC50259.1| (U26644) encodes region of fatty acid synthase activity; FAS; multifunctional protein [Homo sapiens] POS=2329 (SEQ ID NO:23)

G=3728: Mg=1147.5: Tg=48+-1 #E+(3,2) #EST+(2,1) #FR+(5,2) #G+(4,3) #K+(13,6)
S=91(92,90) Mp=1147.7(0.2) Tp= 45 A2=>1k/20 P=KVLDFEHFL genpept PR=>gi|189022|gb|AAA36348.1| (M22920) smooth muscle mysoin light chain [Homo sapiens] POS=28 (SEQ ID NO:193)

G=3743: Mg=1152.6: Tg=47+-0 #D+(5,3) #F+(1,1)
S=79(82,72) Mp=1151.6(-1.0) Tp= 43 A2=>1k/24 P=YLPEDFIRV genpept PR=>gi|2653877|gb|AAB87669.1| (AF026273) interleukin-1 receptor-associated kinase-2; IRAK-2 [Homo sapiens] POS=381 (SEQ ID NO:194)

G=3754: Mg=1156.5: Tg=35+-1 #G+(3,2) #K+(8,5)
S=91(95,83) Mp=1156.5(0.0) Tp= 43 A2=403/28 P=FLSEHPNVTL genpept PR=>gi|5102831|emb|CAB45270.1| (AL022318) bK150C2.2 (Phorbolin 3) [Homo sapiens] POS=107 (SEQ ID NO:195)

G=3806: Mg=1210.4: Tg=42+-1 #E+(7,4) #EST+(1,1) #FR+(5,3) #G+(20,11) #K+(10,6) #L+(4,2)
S=76(80,68) Mp=1210.6(0.2) Tp= 44 A2=128/21 P=LLLDVPTAAVQA genpept PR=>gi|6165618|gb|AAF04618.1|AF097362_1 (AF097362) gamma-interferon inducible lysosomal thiol reductase [Homo sapiens] POS=26 (SEQ ID NO:3) ref

G=3831: Mg=1258.5: Tg=54+-1 #S+(12,2) #E+(12,6) #F+(12,6) #EST+(1,1) #FR+(7,3) #G+(20,10) #H+(1,1) #K+(10,5)
S=87(96,68) Mp=1258.6(0.1) Tp= 58 A2=611/27 P=FLFDGSPTYVL genpept PR=>gi|1049053|gb|AAC50259.1| (U26644) encodes region of fatty acid synthase activity; FAS; multifunctional protein [Homo sapiens] POS=2329 (SEQ ID NO:24)

G=3859: Mg=1360.4: Tg=44+-1 #E+(3,2) #G+(19,10)
S=91(99,75) Mp=1360.6(0.2) Tp= 42 A2=>1k/28 P=ALWDIETGQQTV genpept PR=>gi|306785|gb|AAA35922.1| (M16538) G protein beta subunit [Homo sapiens] POS=167 (SEQ ID NO:25)

DISCUSSION

Among the thousands of different peptides presented within the context of the MHC class-I on cancer cells, only a few may eventually become candidates for the development of anti-cancer vaccines. The identification of such cancer specific peptides depends on sequencing a relatively large number of peptides.

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While reducing the present invention to practice, a novel method was developed to identify candidate peptides for the development of anti-cancer vaccines. The novel method involves expressing the soluble extra-cellular domain of the MHC molecules that are simple to purify and the recovery, from them, large amounts of MHC bound peptides ready for identification by ESI-MS/MS.

Purification of the extra-cellular domain of MHC was previously achieved by truncating its entire transmembrane and cytoplasmic domains [30], by using a non-functional transmembrane domain such as $Q10^b$ [24] or fusing the extra-cellular domains to soluble secreted proteins such as antibodies Fc domains [31, 32]. Such sMHC molecules were produced in cultured cells of murine [33], human [30, 34] or insect [35] and in bacteria [36]. The soluble MHC molecules expressed by the murine or the human cells were capable of binding to their cognate TCRs, indicating the presence of bound authentic peptides that mediate this interaction [33, 37]. Bound peptides recovered from the secreted murine MHC H-2Ld were analyzed by Edman sequencing [38]. More recently, peptides recovered from the murine $Q2/Q10^b$, which is a natural mutation resulting in the formation of soluble and secreted MHC molecules, were analyzed by ESI-MS/MS [39]. The results, however, were very disappointing as only six peptides were recovered from 50 liters of culture medium [39].

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While culture cancer cell lines are invaluable model for cancer research, only a limited number of good model lines are available for the study of tumor immunology since some of the better model cell lines have rare MHC haplotypes or down regulated MHC expression altogether. The introduction of foreign MHC into such cells in accordance with the teachings of the present invention facilitates the use of the desired model cell lines for the search for cancer specific MHC bound peptides. The recovery of secreted MHC from the growth medium helps to sidestep possible interference by the cell's background MHC haplotypes.

The number of peptides identifiable during each ESI-MS/MS run performed in accordance with the present invention was limited by the rate the mass spectrometers can switch between measuring the full spectrum to performing CID, which was about four seconds. Therefore, during a chromatography of ninety minutes, around a thousand different peptides could be mass measured and fragmented. The elution order of most of the peptides recovered for MHC of a particular type and resolved in different chromatography runs was similar. Therefore, their masses and CID data were combined in order to improve their signal-to-noise ratio.

About one thousand different molecules that are certainly peptides have been fragmented at least twice in all the different chromatographs and out of these about two hundreds different peptides have been identified at high certainty. Most of these peptides were derived from housekeeping proteins

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and only a few were derived from proteins related to cancer. To increase the likelihood of identifying more new cancer specific peptides, the total number of identified peptides should be further enlarged. Identification of large number of peptides is currently limited by both the availability of sufficient amounts of peptides, by the capabilities of the mass spectrometers and by the non-completeness of the databanks. With the expected near availability of the entire human genome sequence, it is expected that more of the peptides will be identifiable, excluding mutant peptides that will still need to be sequenced *de novo*.

The soluble and secreted MHC molecules described here present similar patterns of peptides as do the original cell surface MHC. This conclusion emanates from the observation that most of these peptides, posses an amino acid sequence that fit the known sequence consensus of HLA-A2.1 and of B7 (see score columns in Table 8 above). Some of the peptides have been identified previously as MHC bound peptides and thus indicate the validity of the methodology of the present invention. The most significant advantage of the use of secreted MHC as a source for peptides for analysis has to do with the order of magnitude larger recovery of sMHC molecules and therefore peptides per cell over the alternative purification from detergent solubilized cells and the purified sMHC molecules were free of interfering cellular debris and detergents.

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Direct biochemical analysis of peptides eluted from MHC molecules that are recovered from cancer cells, allows unbiased identification of those peptides that are actually presented by the MHC. Even though, identifying putative MHC bound peptides using computer programs based on the consensus motifs followed by synthesizing them and testing their immunogenicity, bypasses the reliance on expensive and technically demanding mass-spectrometry needed for biochemical analysis of MHC bound peptides. However, the motif prediction approach is dependent on the availability of well-established consensus for the MHC allele of interest and is hampered by the difficulty of taking into account the processing machinery involved in generating the peptides and transporting them to the MHC [13]. Moreover, it was suggested that contaminating protecting groups inadvertently left on the synthetic peptides are very immunogenic and may become the target for the activity of the CTLs. The CTLs generated *in vitro* are often low affinity binders and incapable of recognizing the rare peptides actually presented by the cancer cells *in vivo* [10].

The examples of identified peptides listed in Table 8 above include peptides that do not fit the accepted consensus of MHC bound peptides presented by the studied MHC haplotypes. Peptides longer than ten amino acids are not expected to be common among MHC class-I peptides [40, 41]. However, in this study, few peptides of 11 amino acids (p1210, SEQ ID NO:3 and p1258, SEQ ID NO:24) and 12 amino acids (p1360, SEQ ID NO:25) long

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were observed among the identified peptides. The computer programs for motif predictions of class-I peptides are not able to predict such peptides as their length is outside the consensus [42, 43]. The detection of longer peptides among the peptides in the natural mixture suggests that the consensus motif should possibly be extended to include such outliers. Another interesting observation is the relatively abundance of peptides that originated from overlapping parts of the proteins with one or two amino acids difference in length such as p800 (SEQ ID NO:4) and p913 (SEQ ID NO:5) from β-catenin, p1145 (SEQ ID NO:23) and p1258 (SEQ ID NO:24) from fatty acid synthase, p898 (SEQ ID NO:1), p1011 (SEQ ID NO:2) and p1210 (SEQ ID NO:3) from IP30 (Table 8). Moreover, peptides p1080 (SEQ ID NO:19) and p1094 (SEQ ID NO:21) are derived from homologous site in two alleles of the same protein. This observation points to the existence of structural hotspots for generation of peptides, possibly as a result of heath-shock proteins binding and protection from complete proteolysis of these regions. Differences in length could also result from incomplete trimming of the peptides in the endoplasmic reticulum [44, 45].

Interesting observations are the large similarities between the patterns of peptides produced by cell lines of different tissue origin and on the other hand, the presence of a few peptides that are unique to one type of cancer cells. The ability to characterize the similarities and differences between peptide patterns of different cell lines and growth conditions and between different

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HLA haplotypes are among the most important possible uses of the novel methodology presented herein.

The most effective mean to ascertain the identity of the amino acid sequences of peptides that were identified by this method is to compare their retention times, their exact masses and their CID data to those of the corresponding synthetic peptides [16, 39, 46, 47]. The sequences of all the peptides that were identified at high confidence by searching the databank with their mass spectrometry data were shown to be correct when these parameters were compared with the corresponding synthetic peptides.

A number of peptides identified here were derived from known tumor antigens. Those peptides that attracted the attention as possibly cancer specific were chemically synthesized and tested again. The fact that a few of them elicited a CTL response in mice may point to their possible immunogenicity in human.

Tumor proteins from which identified peptides were derived included mucin (MUC1), a well-studied tumor-associated antigen that is up regulated in breast and ovarian carcinomas [48]. A number of HLA-A2.1 restricted MUC1-derived CTL epitopes were identified by the motif prediction approach [26, 49-52]. Peptide p947 (NLTISDVSV, SEQ ID NO:8) identified here from breast carcinoma cells (MCF-7) is the same peptide that was predicted and confirmed to be a HLA-A2 antigen originating from MUC1 by Carmon et al. [26].

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Another peptide derived from a known tumor antigen, was p1091 (SEQ ID NO:20) from the testis-cancer antigen MAGE-B2. It belongs to a group of 21 known genes that are essentially silent in most normal cells except for testis and placental trophobalsts and since different member of the MAGE proteins are expressed in a variety of tumors, they attracted significant attention as cancer vaccine candidates [53-57]. A few peptides were identified so far from the MAGE proteins by genetic approach and by predicting their sequence based on the known motifs rather than by the biochemical approach [27, 28, 58-61] (reviewed in [10]). The identification of the novel MAGE-B2 derived peptide p1091 (GVYDGEEHSV) (SEQ ID NO:20) by the direct biochemical approach is a very encouraging observation that confirmed the validity of this method for identification of novel tumor specific antigens. Homologous peptides from MAGE-A4 and MAGE-A10 proteins were previously identified as MHC bound peptides and tested for their immunogenicity (see Figure 4D). This suggests the existence of a possible hot spot within the MAGE protein for processing as MHC bound peptides [27, 28].

Peptides derived from other proteins that are involved with cancer progression and may also serve as candidates for anti-cancer vaccines of diagnosis include p913 (SEQ ID NO:5) from β-catenin, which is normally involved in cellular adhesion, signal transduction and as a transcription enhancer with a possible oncogenic role in colorectal cancer. Abnormal high amounts of the protein were found in the cytoplasm in cancer cells instead of

the intracellular boundary in normal cells and this abnormal behavior was correlated with metastasis [62-64]. Peptide p1145 (SEQ ID NO:23) and p1258 (SEQ ID NO:24) is derived form fatty acid synthase (FAS), a biosynthetic enzyme expressed in liver and lactating breasts and is a marker of poor prognosis when expressed in colon, prostate, ovarian, breast and endometrial cancers. Its significance for cancer is was established by inhibiting it activity, which leads to apoptosis in cancer cells [65-69]. The enzyme DNA methyl transferase (MTDM) is the source protein for p1028 (SEQ ID NO:13) an enzyme that is highly expressed in different cancer cell types, including prostate and breast [70-72]. Increased MTDM activity is usually associated with tumor progression and is considered to be an important event in cell transformation [71, 73].

Once tumor specific MHC bound peptides are identified and their ability to stimulate an immune response is demonstrated, such peptides become candidates for adoptive immunotherapy. Identification of peptides originating from normal proteins that are uniquely expressed in non-vital organs, such as breast, prostate and ovaries can become very useful for immunotherapy of these cancers. The potential usefulness of identified immunogenic peptides should be evaluated by the presence of specific T cells directed against them in patients inflicted with the particular cancer using standard assays such as ELISPOT and CTL. The assay of immunizing mice with the peptides described herein was meant to serve first as validation that

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these peptides are indeed MHC bound peptides with affinity for the HLA-A2.1 and as the preliminary indication of their immunogenic potential.

Secreted soluble MHC such as described herein can also be used for analysis of peptides presented by cells involved with pathologies other than cancer, such as autoimmune diseases and viral infections with the aims of identifying peptides of significance for treating these diseases. The method can also be used for identifications of MHC bound peptides presented on normal cells of specific tissues, peptides presented by particular MHC alleles and peptides originating from expression of particular proteins of interest. Moreover, the approach can be used for analysis of MHC bound peptides derived from over-expression of specific proteins, from induced mutations, as a result of metastasis progression and as a way for searching for peptides derived from signal peptides of cell surface proteins. The approach described in this study is also useful for comparisons between patterns of MHC bound peptides induced by minor changes in the cells growth conditions such as the addition of hormones, the expression of a foreign protein or under stress conditions.

Therefore, an appealing outcome of the methodology described herein is that the simple expression of different recombinant MHC molecules in different cell lines in a soluble, secreted form and their easy recovery from the growth medium with their peptides still attached, followed by comprehensive analysis of the peptides may become a good staging point for above listed

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ambitious research projects. Such 'human MHC-peptide projects' are worthy goals to follow the human genome and proteome projects.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

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